A Summary of Current Program 7/1/67

and Preliminary Report of Progress

for 7/1/66 to 6/30/67

NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1966, and June 30, 1967. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

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TABLE OF CONTENTS

INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, grain sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

The scientific research effort at the Northern Division amounts to approximately 183 scientist man-years. In addition, the Division supervises domestic research contracts equivalent to 25.3 scientist man-years and grants equivalent to 20.0 scientist man-years, and sponsors a comprehensive program of research comprising 35 PL 480 grants to foreign institutions.

In this report, utilization research of the Northern Division is discussed under the 17 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and grants and sponsorship of related research performed abroad under grants of Public Law 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1966, through June 30, 1967.

Also for each area, the size of the related program maintained by State Experiment Stations is given. In addition to examples of recent utilization research accomplishments of the Northern Division, examples of achievements by the State Stations are provided.

Survey of U. S. grains shows little aflatoxin contamination. The presence of aflatoxin in moldy peanut meal was responsible for the deaths of 100,000 turkey poults in England in 1960. Later, aflatoxins were shown to be carcinogenic for some experimental animals. These discoveries dramatized the need for more information on the presence of aflatoxins in feed and food grains in the United States. Accordingly, Department scientists made a survey for aflatoxin, testing 3,548 samples representing all USDA Grain Inspection Service grades of corn, wheat, oats, soybeans, and grain sorghums collected at selected terminal markets from October 1964 to November 1966. To assure maximum reliability of results, the number of samples of each kind and grade of grain was chosen by a statistical design which took into account the relative volume of shipments. A very low incidence of aflatoxin was found in these grains except in the lowest grade, i.e., sample grade. Even in this grade only 4 percent of the samples contained aflatoxin. Some of the reasons for placing grain in Sample Grade are moldiness, mustiness, off odor and off color. The results point up the importance of keeping this grade separate and not using it in foods and feeds.

Specifications developed for corn-soya-milk (CSM) blended food product. Corn-soya-milk (CSM) blended food product, a balanced food intended as a dietary supplement for children, was developed cooperatively by industrial and Department scientists for donation overseas through programs of voluntary agencies, AID, and UNICEF. Department scientists conducted extensive processing, storage, analytical, and organoleptic tests on CSM which resulted in specifications for all purchases. These specifications are the basis for the official inspection and insure a product having uniform quality and characteristics. As of May 1967, over 230 million pounds of CSM have been distributed in 90 foreign countries.

Potential new sources of vegetable proteins. The most comprehensive survey ever made of seeds as protein sources has been conducted by Department scientists. Of seed from 4,300 plant species, 535 had more than 35 percent protein. Complete amino acid analyses by precise modern procedures have been made on 380 species to determine nutritional value. This is over four times the total number of species for which reliable amino acid values were available prior to 1957 when this survey program was started. Seed protein of some species contained amino acids in amounts that suggest good nutritional balance. Others, while not themselves balanced, were rich in amino acids that are too low in present vegetable foods and feeds. The data offer a basis for suggesting potential new crops for field trials, feeding tests, and for study of adaptability to the agricultural economy of particular regions in the U. S. Such considerations and experiments point the way for promising new feed and food crops to supplement the world shortage of dietary proteins.

Oil well drilling fluids based on polysaccharide gum from cereal starches. Polysaccharide B-1459 gum has been selected by industry as the base component for a new patented oil well drilling mud system. This gum is produced by four industrial companies using the process developed by the Department based on bacterial fermentation of cereal starches. About 2 pounds of starch are used to produce 1 pound of gum. B-1459 solutions in water have unusually high viscosities that are stable over wide ranges of temperatures, salt concentrations, and alkalinities--conditions normally encountered in oil well drilling. Over 20 use patents for the gum already have been obtained by industry. Seven major mud producers have licensed and are using the new drilling fluid. This B-1459 market already exceeds a million pounds annually and is predicted to continue its rapid growth.

New catalyst for producing improved edible soybean oil products. A copper-containing hydrogenation catalyst has been developed by Department scientists for use in production of edible soybean oils having improved flavor stability. They also discovered that after a special activation process a commercial copper catalyst was highly effective in treatment of soybean oil with hydrogen for selective removal of linolenate, the unstable component of the oil. Salad oils containing less than 1 percent of linolenate will be easily produced with the aid of the new catalyst. At the same time, most of the nutritionally desirable linoleate will be retained. Since no solid fats are formed, the added cost of winterizing to remove hardened products will be eliminated. "Olive oil" type cooking oils will result from more drastic hydrogenation treatment. The new catalysts are being tested by commercial salad oil producers. The treated oils are expected to have the stability needed to withstand storage and transportation for foreign marketing. Possibilities are, therefore, substantially enhanced for soybeans to play a major role in overcoming the annual world food deficit of 4 billion pounds of food fats and oils.

Wheat flour used as paper size by industry in the Pacific Northwest. Wheat flour fractions modified by a process developed by Department scientists have been used as an additive by the paper industry in the Pacific Northwest. The process developed involves the enzymatic conversion of the flour to a desirable form, is simple to operate, and is readily applicable in existing paper mills. The process works with various types of flours, but best results are obtained with flour from soft wheat. Wheat produced in the Pacific Northwest is practically all soft wheat. The paper industry is also concentrated in this region. Thus, a market is available for industrial grade, regionally produced flour that can compete with other commercial products currently used as paper size, practically all of which must be imported or obtained from other regions of the United States. At least three industrial companies have used the flour on a plant scale.

New industrial starch developed. Through the cooperative effort of Department scientists, corn breeders, university personnel, and industry, corn starches containing various amounts of amylose are now commercially available. Two starches are now marketed, namely, one containing 50-60 percent

amylose and one containing about 70 percent amylose. The starch in regular yellow dent corn contains only about 27 percent amylose. Control of the amylose content provides new and diverse markets. In general, the film-forming properties of starch improve in proportion to its amylose content. Growing industrial markets for the new starches are found in the manufacture of glass fibers and paper products. Other potential uses include edible coating for foods. Three large industrial corn-milling companies are now processors of the corn grown under contract by farmers.

Guidelines for corn drying adopted by industry. Mechanical harvesting and shelling of corn in the field has resulted in major cost savings but has generated a need for controlled drying of the corn. Research by Department scientists has assisted in the establishment of the most suitable drying conditions for the corn to prevent damage that impairs its feeding value, or reduces its value for dry- and wet-milling operations. Included was the development of a simple color test for determining proper drying. This information has been widely used.

In the Corn Belt, up to 60 percent of the corn now is mechanically harvested and field shelled. Maximum efficiency requires that the corn be harvested immediately after maturity. The corn at this stage contains over 25 percent moisture and cannot be stored without spoilage. Special handling, including artificial drying, is required. Annual corn production is about 4 billion bushels with a farm value of over $5 billion.

Fermentative production of mannitol from corn sugar. Department scientists have developed a fermentation process for converting corn sugar into mannitol using a selected strain of the mold Aspergillus candidus. There is a considerable commercial demand for mannitol for use in the pharmaceutical industry as a coating agent for pills. The sweet taste of mannitol masks the taste of drugs, such as aspirin. Because of low tendency to absorb water it is also used as dusting agent for products which may tend to stick together, such as chewing gum. Mannitol is currently made by the chemical reduction of invert sugar derived from cane sugar; however, sorbitol is formed at the same time and the separation of the two is difficult. The Department's process avoids separation problems and is now ready for pilot-plant evaluation. Preliminary cost estimates indicate that it should be possible to compete successfully with chemically produced mannitol.

Village Process for edible soy flour demonstrated in Brazil. Department engineers have developed a "village process" for use in developing countries for making high-grade, full-fat soy flour for food use. The equipment is hand operated and may be purchased for $280. By this process, 300 pounds of flour can be produced in an 8-hour day. These 300 pounds supply half of the daily requirement of protein for 1,600 adults.

After a visit to the Department by the Director General of the National Children's Bureau, the Brazilian Ministry of Health acquired equipment

for the "village process" through UNICEF and asked the Department to demonstrate the process and to provide technical assistance. Two sets of processing equipment have been delivered and four additional sets are on order for different areas of Brazil. A Department engineer went to Brazil to give instructions on operation of the equipment to staff members and groups of doctors, nutritionists, and social workers of the National Children's Bureau, Ministry of Health, of Brazil. These groups in turn will train task groups to operate the process in the interior parts of Brazil for the purpose of improving nutrition among young children suffering from dietary protein deficiency.

Identity-preserved soybeans marketed for Oriental foods. United States-produced soybeans are finding increased markets in Oriental foods, resulting from a research study by Department scientists in cooperation with Japanese workers. Solutions have been found to the previous objections to U. S. soybeans such as uneven cooking, dark-colored products, and undesirable flavors. Processing techniques, evaluation procedures, and the selection of U. S. soybean varieties that give products with specific tastes and flavors desired by foreign users have resulted in shipment of increasing quantities of identity-preserved soybeans to Oriental countries. These U. S. soybeans are now preferred to those grown by the countries themselves, or imported from countries such as Mainland China.

At least three companies are now exporting several million bushels annually of identity-preserved beans. This is a growing cash market. For example, in Japan where 40 percent of the soybean consumption is used to produce traditional soybean protein foods, U. S. imports have increased from 40 million bushels in 1962 to 65 million in 1966.

RECENT ACCOMPLISHMENTS OF STATE AGRICULTURAL EXPERIMENT STATIONS

Colorado

Thin flaking of corn for cattle feed. The proven superiority of flaked corn over other conventional methods of processing feed grains has led Colorado station workers to study the degree of thinness or thickness most suitable for cattle feeders. Comparison of "thin" flakes (1/32-inch) with "thick" flakes (1/12-inch) in heifer feeding trials of 163-day duration demonstrated a greater feed efficiency and a cheaper cost of gain favoring "thin" flakes. Average daily gain was 2.82 for thin flakes contrasted with 2.70 pounds for thick flakes, whereas mash-fed animals showed gains of only 2.65 on control diets with finely ground corn passing a 1/4-inch screen. At a selling price of $25 per cwt. final weight and with lowered feed costs plus increased returns owing to greater gains, a total advantage of $8.13 per head accrued for animals fed thin flakes. These significant savings resulted from adjusting the tightness setting of the rolls used for flattening steamed corn to produce flakes having a 1/32-inch thickness after drying to 12 percent moisture.

Indiana

Carbohydrate reactions with chlorine. Carbohydrate chemists at the Indiana station have continued their series of basic studies on the reactions of chlorine with oligosaccharides and with simpler sugar models. From this work is emerging an understanding of the starch and cellulose bleaching processes used for wheat flour and paper pulp. Information on the mechanism of chlorinolysis reactions has been useful in structurally explaining and predicting glycoside bond cleavage, starch oxidation, depolymerization of polysaccharides, and the small but significant transformation of glucose to galactose-units in the pyranose configuration. Earlier Indiana work has differentiated the role of moisture levels on chlorine treatment of aqueous, semi-dry, and dry starch and the effects of light and dark. Recent evidence for the formation of a hypochlorite ester as a key intermediate has been supported by observations with amylose.

Kansas

Water-soluble pentosans in flours. In a study designed to correlate the composition of wheat flours with functional properties in breadmaking, Kansas station scientists, in cooperation with USDA, have determined the role of the water-soluble pentosans in flours. Pentosan preparations from the durum flour differed from the preparations from the other flours in carbohydrate composition, electrophoretic mobility of associated proteins and infrared spectra. Pentosans from hard red winter, hard red spring, soft red winter, and club--but not durum--increased water absorption. Adding pentosans increased oxidation requirements. Loaf volumes were increased by adding pentosans from hard red winter or club flours, and were decreased by adding pentosans from soft red winter and durum flours.

Nutritive value of sorghum grains. Differences in the protein content of sorghum grains may affect the nutritive value of poultry diets and thus may be regarded as an important quality factor in feed formulation. Although the amino acid composition of the sorghum proteins was similar in sorghum grain samples containing 8, 10, and 12 percent protein, research workers at Kansas State showed that sorghum grains of high protein content produced significantly more gain than low protein grain when substituted for corn in rations containing a constant level of soybean meal. Calculated limiting amino acids in the diets were methionine and glycine, with arginine and lysine next most limiting. Chick performance was not affected by protein levels when the grain was formulated into diets of equal protein.

Louisiana

Irradiation pasteurization of soy flour. Commercial application of low dose gamma radiation for the preservation of large quantities of soy flour has been investigated by Louisiana station scientists. The researchers concluded that the cost of irradiation is relatively inexpensive when compared to the value of extending the storage life of soy flour. Chemical, microbiological, physical and organoleptic tests verify the potential. Quality was retained for periods of time in excess of 56 days after irradiation.

Michigan

Identification of soybean components. Soybean meal contains an unidentified substance responsible for improved growth of turkey poults. Seeking to characterize the unknown feed component, research scientists at the Michigan station have fractionated soybean flakes and meals, and their results suggest that an amino or hydroxy group in the substance is essential for its biological activity. While the activity can be extracted from unheated soybean flakes by water or from heated soybean meal by methanol, the reverse is not true. The growth factor can be reacted with acetic anhydride and rendered biologically inactive, however alkaline hydrolysis will restore the growth promoting activity. Although the primary factor responsible for improved growth of turkey poults is organic in nature some activity is found in the ash of the methanol extract, which indicates that an inorganic element not now recognized as essential may promote growth.

Montana

New starches from Saponaria. Starch has been produced from Saponaria vaccaria (cow cockle) by a modified wet-milling process. Utilization research at the Montana station with agronomically promising varieties of cow cockle seeds has led to the isolation of substantial amounts of a starch for studies on its commercial potential. This unusual starch is made up of extremely small granules (about 1 micron in size) with an apparently very heterogeneous structure. It has pasting characteristics similar to potato starch and swelling power resembling rice starch. Cow cockle starch in water possesses stability on cooling and may have application advantages where freeze-thaw stability is important as in waxy sorghum. The new starch may have numerous uses as dusting starch in cosmetics and industrial dusting starch. It is also of theoretical interest for fundamental studies owing to its unusual paste properties.

Fragmentation during enzymatic degradation of cellulose. The nature of cellulose breakdown in forage digestion has been explored by Virginia station scientists. They have shown with purified cellulase that the enzymes solubilize cellulose not only by erosion of the particle surfaces but also by fragmentation of the larger cellulose particles into sub-units which are still particulate. Tighter protein hydrogen bonding to cellulose has been suggested as the mechanism which breaks the cellulose hydrogen bonds formerly holding the initial micelles together.

AREA NO. 1: CORN UTILIZATION
INDUSTRIAL PRODUCTS

Problem. About 3 billion pounds of cereal starches and flours are used annually in the U. S. for industrial purposes. Corn is the source of most of these products. Industrial outlets for starches and flours are, however, constantly threatened by synthetic products derived from nonagricultural sources. Maintenance of the present and future competitive position of corn starch and flour in industrial markets requires a continuing program of basic and applied research.

The most promising outlets for new and improved industrial products derived from corn include the paper industry, industrial chemicals, adhesives, protective coatings, plastics, elastomers, and thickening agents. The greatest opportunity exists in the manufacture of paper and paperboard products, the U. S. production of which is over 40 million tons per year and growing at a considerably faster rate than is population. Not only the total volume of starch consumed in the paper industry but also the average amount used per ton of paper product has increased very substantially since 1950. This favorable picture stems from research conducted in the past. To maintain or, more desirably, to increase the utilization of corn starch and flour in competition with synthetics, new concepts must be evolved that relate chemical modification of starch with specific properties imparted to paper products. Technology must be developed to establish optimum procedures for industrial use of promising products currently under study such as starch xanthates, starch graft copolymers, cyanoethylated and sulfated starches, and the new starch from high-amylose corn.

Industrial chemicals provide a multibillion-pound annual market in which starch-derived products should share to an increasing extent. At 5 to 6 cents per pound, corn starch is an attractive raw material for the manufacture of industrial chemicals. When fermentative, rather than conventional chemical, conversion is applicable, even cheaper sources of starch such as flour and ground whole grain can be used as the raw material. Over a billion pounds of corn sugar is used annually in the manufacture of such well-known industrial chemicals as sorbitol, mannitol, citric and gluconic acids, and methyl glucoside. Promising leads requiring research to insure successful future developments include nitrogen, sulfur, and unsaturated derivatives of starch, vinyl glucosides, industrial enzymes, and enzymatic starch conversion products such as oligosaccharides, polyols, and glucosides.

Adhesives represent a field long dominated by starch, which accounts for nearly half of the annual 2-billion-pound market for these products. However, competition by synthetic resins is especially vigorous and effective because of the specialized properties required to achieve increased production of products like corrugated container board on automatic

machinery. The overall growth rate of adhesive consumption is almost 7 percent per year. Since starch is usually cheaper than synthetic adhesive resins, prospects are good for meeting the competition through research designed to improve viscosity properties, bond strength, tack, and drying time of starch-derived adhesives.

The remaining outlets--coatings, plastics, elastomers, and thickeners--represent a multibillion-pound market in which starch-derived products having suitable properties should find ready acceptance. Microbial polysaccharide gums, starch graft copolymers, and urethane foams from starch-derived polyols typify the products that result from research on corn starch and flour.

Research oriented specifically toward particular industrial applications of final products must be founded on a vigorous and wide-ranging program of basic and exploratory investigations. Such studies lead to the discovery of new concepts, principles, and reactions that are the source of new processes and products for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of corn, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of corn totals 59.3 scientist man-years. Of this number, 10.0 are devoted to chemical composition, physical properties and structure; 18.2 to chemical and physical investigations to improve products; 20.7 to microbiology and fermentation; and 10.4 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (8.4 scientist man-years) involves study of starch, amylose, amylopectin, proteins, and lipids of corn. A portion of the work is related to problems pertinent to high-amylose corn. During the year, studies on the rheological properties of starch were initiated. Grants (1.6 scientist man-years) are in effect with Iowa State University, Ames, Iowa, for basic research* on heat, mass, and momentum transport of cereal starches and flours; Purdue Research Foundation, Lafayette, Indiana, for research* on the effects of disulfide bond cleavage on the structure of corn and wheat endosperm proteins; Arizona State University, Tempe,

*Work covers more than one commodity; only effort allocated to corn is included in total.

Arizona, for basic investigations of the helical structure of amylose; and the State University of New York, Syracuse, New York, for investigations of starch fine structure. Grant research was completed by the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for basic studies on variations in starch granules of genetically different corn samples and by Princeton University, Princeton, New Jersey, for basic research on the relationship of viscoelastic properties of amylose film to structure and function of plasticizers.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (12.2 scientist man-years) is directed to wide-ranging study of the chemical reactions of starch with the objective of discovering new chemical products and processes having potential for industrial use. During the year, studies on starch-based plastifoams were completed and research on related noncellular plastics was initiated. Research contracts (2.5 scientist man-years*) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of acetylene with methyl glucoside; with the University of Akron, Akron, Ohio, for evaluation of starch and starch derivatives as reinforcing agents for natural and synthetic rubber; with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides; with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper; and with General Mills, Central Research Laboratories, Minneapolis, Minnesota, for studies on the development of cereal proteins having utility as flotation and flocculating agents. Contract research was completed by The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media, and with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers. Grants (3.5 scientist man-years*) are in effect at Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; at Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; at Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur; at the University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; at the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides; and at Southern Illinois University, Carbondale, Illinois, for studies on the alcoholysis of carbohydrate esters.

Research on microbiology and fermentation conducted at Peoria, Illinois, (16.4 scientist man-years) includes studies on the use of microorganisms

*Work covers more than one commodity; only effort allocated to corn is included in total.

to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. A large collection of pure cultures of industrially and agriculturally important microorganisms is maintained. The Pioneering Laboratory for Microbiological Chemistry conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (1.2 scientist man-years*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation* of morphological changes involved in sporulation; at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective; and at Michigan State University, East Lansing, Michigan, for investigation of the biochemical properties of variant cultures of Bacillus popilliae. Contract research at the Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms has been completed. Grants (3.1 scientist man-years*) are in effect with Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; Iowa State University, Ames, Iowa, for investigation* of bacterial amylases and their action patterns; the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch; the University of Nebraska, Lincoln, Nebraska, for structural studies of fungal glucohydrolases; Baylor University, Houston, Texas, for cytology of ascospore formation in yeasts; the University of Minnesota, Minneapolis, Minnesota, for studies of cellular differentiation and physiology of selected molds; East Texas State University, Commerce, Texas, for determinations of branching in polysaccharides; and Indiana State University, Terre Haute, Indiana, for surveys of gum-producing microorganisms. During the year, grant research was completed by Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes.

Research conducted at Peoria, Illinois, on technology--process and product development (8.1 scientist man-years) is concerned with detailed study and evaluation of starch derivatives having definite potential for industrial utilization and of processes for making them. Research contracts (2.3 scientist man-years*) are in effect with Stanford Research Institute,

*Work covers more than one commodity; only effort allocated to corn is included in total.

Menlo Park, California, for process development of selected starch graft copolymers; with Western Michigan University, Kalamazoo, Michigan, for evaluation of modified cyanoethylated starches for applications in paper; with Battelle Memorial Institute, Columbus, Ohio, for studies on starch derivatives for use as colloids in water-emulsion paints; and with Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations on the use of starch glycosides in coatings and plastics. During the year, contract research on starch and other cereal grain xanthides was completed by Battelle Memorial Institute, Columbus, Ohio.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds.* Research on chemical composition, physical properties and structure involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); to the University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968). During the year, research on glucopyranose rings in starches and dextrins was completed at the "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy.

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); Institute for Fibres and Forest Products Research, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968); and to the University of Graz, Graz, Austria, for rheological studies on aqueous dispersions of modified cereal starches and paper coating formulations containing starch-based adhesives (3 years, 1966-1969). During the year, research was completed on phosphorus- and sulfur-containing cationic starches at the National Institute of Technology, Rio de Janeiro, Brazil.

Research on microbiology and fermentation involves grants to the University of Allahabad, Allahabad, India, for studies on survival of lyophilized

*Effort prorated among corn, wheat, and grain sorghum.

microorganisms (5 years, 1962-1967); Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India to find new accessions for the ARS Culture Collection (5 years, 1965-1970); the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and of mevalonic acid (3 years, 1965-1968); the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969). During the year, research was completed on collection of new Mucorales species at the University of Allahabad, Allahabad, India; on investigations of sugar phosphate derivatives in molds at the University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England; and on studies on the preparation and characterization of dextran derivatives at the University of Rome, Rome, Italy.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.6 scientist man-years is devoted to research on industrial and feed uses of corn.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. High-amylose (HA) corn development. Studies on structural and chemical differences in corn genotypes show that the basis for high lysine content of certain opaque and floury mutants is greatly reduced deposition of low nutritional quality zein protein and concomitant increase in deposition of glutenin and globulin, both of which are relatively high in lysine and other basic amino acids. A new type of zein need not be postulated to explain the altered protein composition. During the past year, 14,900 amylose analyses have been completed for the cooperating plant breeders working on the development of high-amylose corn. Of these samples, 204 assayed 85 percent apparent amylose or higher. The highest sample assayed at 87.9 percent apparent amylose. Work on improving and simplifying the amylose analysis continues with studies on the determination of amylose in dimethyl sulfoxide extracts of high-amylose corn. In analyzing the whole corn kernel for amylose rather than only the endosperm protein, it was found that the oil from the germ appears to have no effect on the amylose determination in DMSO extraction method. Most recent evaluation of error of DMSO method shows a standard deviation of $\pm$ 1.2 percent amylose for corn with about 80 percent amylose.

2. Chemical studies on corn components. A general method for isolation of minimally degraded corn starch was developed. It involves low temperature

dispersion with either concentrated salt solutions or 90 percent DMSO and the use of enzymes to remove bound protein. This procedure consistently yields amylopectin having a very high molecular weight ($\bar{M}_w$, 6.6 X 10^8). In aqueous solution, amylopectin obtained from 70 percent high-amylose (HA) corn displayed retrogradation, whereas comparable fractions from dent or waxy corn starch did not; the amylose fraction from HA corn also differed in retrograding more easily than amylose from dent corn. Stable amylose fractions of dent corn were prepared in concentrated salt solutions. It was found possible to proceed from a DMSO dispersed sample to both the BuOH-amylose complex and a 4 M guanidinium hydrochloride (GHCl) amylose solution and from the BuOH complex to the 4 M GHCl solution. No residual DMSO or GHCl was found with the amylose after conversion to the butanol complex and precipitation in EtOH-MeOH solution.

Grant research at the Arizona State University on reactions of helical amylose with small molecules shows that at pressures above 525 mm., HCl degrades amylose, whereas below this pressure the "V" crystal lattice is unaffected. Crystallographic and chemical considerations of the crystal structure of the KBr-amylose addition compound indicates that the correct space group is $P4_32_1$. The three dimensional Patterson map revealed peaks which were assigned as Br-Br and KBr interactions for Br^- situated at (.20, .20, 0) and K^+ at (.54, .54, 0).

Studies under a grant to Purdue University on disulfide bond cleavage in wheat and corn proteins has revealed that the increased lysine in opaque-2 corn is due to a high content of globulin and glutelin but not to a change in zein composition. Work continues under this grant on the development of extraction procedures for recovering the various proteins from opaque-2 and dent corns.

A micromethod has been developed at the University of London, London, England, under a PL 480 grant for the determination of the chain length of glycogen and amylopectin which requires the simultaneous and complete degradation of the polysaccharide with β-amylose and pullulanase to maltose and glucose. The specific determination of glucose permits the calculation of a chain length. The reliability of the method has been confirmed with glycogen and amylopectin samples whose chain length had been determined by periodate oxidation. Considerable effort has been expended to verify the enzymatic purity of the β-amylose and pullulanase used. The β-amylose obtained commercially contained variable amounts of a maltose which could be partially removed by gel filtration on Sephadex G-100 or suppressed by erythritol. The pullulanase, an acetone powder of the extracellular medium of a culture of Aerobacter aerogenes grown on maltose is enzymatically pure. However, gel filtration on G-200 Sephadex has removed inactive contaminants, purified the enzyme 16-fold, revealed the presence of two active molecular sizes, 100,000 and 50,000, which are interconvertible. Further developments in this project will be limited by its early termination, September 30, 1967.

3. Physical studies on corn components. In NMR studies on grain constituents and grain-derived products, properties of the fungal metabolite, ramulosin, were investigated using IR and ultracentrifugation in addition to NMR. Comparison of ramulosin with several other much-studied β-diketones suggests that one of the stable forms might be an enol-dimer. The integrated optical density at 1,600 cm^{-1} is linearly related to the calculated monomer concentration obtained from NMR data. Ring vibrations of the dimer would be very weak in the infrared since they are symmetrical. Therefore, raman spectra have been obtained on two concentrations of ramulosin in $CHCl_3$. After these data are analyzed, the results will be compared with the calculated dimer concentration. Utility of NMR techniques in structural and mechanism problems should be increased by computer programs currently being devised to assist in the analysis of complicated spin systems.

A complete crystallographic characterization was successfully obtained by X-ray analysis of oriented films of amylose made from the amylose-DMSO complex. The molecular configuration is helical, with six glucose units per turn of the helix and a repeat distance of 8.1 Å along the c axis. The helices are packed in a square array with $a_o = b_o = 9.1$ Å. Symmetry is orthorhombic. This structure has been named "V_{DMSO}." Optical rotatory dispersion studies on amylose films show that there is a direct correlation between structure of amylose in the solid state and its conformation in solution. The specific rotation of amylose V_{DMSO}, amorphous, and alkali films is the same as that of amylose in corresponding solutions of DMSO, 0.5M KCl and alkali. Tests at 16 and 50 percent relative humidity showed that folding endurance of oriented and amorphous films was not changed; however, oriented films had 25 percent greater tensile strength. Photomicrographs and X-ray patterns showed that when films are cast from aqueous dispersions, particles having the "B" structure stick together to form a highly strained film that is brittle at low R.H. When films are cast from DMSO dispersions, crystallization occurs after the film has formed from amorphous amylose.

Under a grant at Princeton University, now completed, modulus-temperature curves were obtained for amylose plasticized with DMSO. The results of these measurements, together with X-ray diagrams, showed that amylose exists as a semi-crystalline polymer in films containing 40 percent or less DMSO. As DMSO increases, crystallinity is lost. The glassy modulus was not affected by incorporation of DMSO, but the rubbery modulus and glass transition temperature (T_g) were depressed.

At Iowa State University, studies under a research grant showed that stirring wheat flour very slowly made air fluidization of the flour possible without the necessity of adding fluidizing agents. Stirring had about the same effect on the behavior of the bed as fluidizing agents except that stirred bed expansion and entrainment are much smaller. Studies on sorption and desorption of water by thin layers of starch showed that desorption took place more slowly than sorption. Sorption rates were observed to be so rapid that 90 percent of equilibrium sorption was reached in 15 seconds.

Rate of sorption of water by starch is affected by the drying conditions and relative humidity of the air. The diffusivity of water vapor is ca. 1×10^{-8} sq. cm./sec. Thermal conductivity of starch is close to that of carbon tetrachloride.

Research conducted under a PL 480 grant at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, extends and augments NU research on amylose and model compounds in aqueous solution, with emphasis on conformation in DMSO (dimethyl sulfoxide) solution. Infrared and nuclear magnetic resonance studies have shown amylose and model compounds including cyclodextrins dissolved in DMSO to have the C1 chair form in solution. Evidence is presented showing the probability of O_2H-$O_3'H'$ hydrogen bonding in DMSO and the stabilization of the helix which this would bring about. A most thorough characterization of cyclodextrins and oligosaccharides, as well as amylose, by IR and NMR has been made including measurements of dichroism in the IR. Three analytical methods were developed in the course of this work: (a) determination of the anomeric configuration and percent anomers of reducing pyranoid sugars; and NMR method based on the magnitude of the H_1H_2 coupling as an indication of the dihedral angle; (b) determination of the composition of mixtures of α- and β-cyclodextrins; an NMR method based on differences in the spectra in the 4.0-4.7 tau region; and (c) determination of the degree of substitution of partially O-methylated amylose and dextrins; an NMR method relating the area of the OH signal to the C_1H signal. This research was completed during the year.

Studies at the University of Osaka Prefecture, Sakai, Japan, showed that a reaction of glucose with o-phenylenediamine gave a quinoxaline derivative that can be determined polarographically. Reaction conditions are being sought for making this method of derivatization applicable to the determination of carbonyl groups in starch and its reaction products. This work is being conducted under a PL 480 grant.

B. Chemical and Physical Investigations to Improve Products

1. Reactions of maltose and glucose. A simplified method has been developed for preparation of anhydrous, non-hygroscopic, high-melting, heat-stable, crystalline maltose. The method involves merely heating the common β-maltose hydrate in a little solvent at 90-100° C. Fifteen different organic solvents and water were used to produce 80-99 percent yields of maltose that contained from 70-83 percent of the more heat-stable α-anomer. Subsequent extractions increased the α-content to 95 percent and gave a new form of maltose with a melting point of 191-193° C. This work suggests the possibility of producing anhydrous α-maltose industrially by heating, seeding, and drying a concentrated maltose sirup. Studies of complexing maltose with ureas showed that an equilibrium exists between complexed and uncomplexed carbohydrate. For several ureas tested, the extent of complexing with maltose decreased in order: urea, monomethyl urea, N,N'-dimethyl urea, cyclohexyl urea. L-arabinose, D-galactose, D-mannose, D-fructose, and methyl α- and β-D-glucopyranosides did not form

an isolable complex. Further studies have shown the importance of the solvent used in forming and precipitating the complex. Both 1:1 and 2:1 maltose-urea complexes have been isolated by use of different combinations of solvent. Structure of a new maltose heptaacetate has been determined to be 1,2,6,2',3',4',6'-hepta-O-acetyl β-D-maltose; the hydroxyl group at C3 was not acetylated by pyridinium acetyl chloride in toluene.

2. New derivatives of starch and related carbohydrates. Experiments on halogen derivatives of carbohydrates have shown that the acid-catalyzed condensation of chloroacetaldehyde diethyl acetal and D-galactitol, D-mannitol, or D-glucitol yields in each case a bis(chloroethylidene) derivative. Characterization of the galactitol derivative showed that the two chloroethylidene groups were linked to the carbohydrate at the 1,3 and at the 4,6 positions, respectively. These derivatives displayed unusual stability to acid conditions. The acid-catalyzed condensation of bromoacetaldehyde with D-mannitol gave a crystalline product which was separated by extensive purification into a high and a low melting isomer of di-O-bromoethylidene-O-mannitol. Although the infrared spectra of these isomers were nearly identical, the di-O-p-tolysulfonyl and di-O-acetyl derivatives of the isomers were different, indicating that the isomers are not different crystalline modifications of the same compound. This particular reaction does not follow the Hann and Hudson rules for predicting structure of the polyolacetal derivatives.

In the contract research at Southern Illinois University, the following steps in synthesis of C4-modified maltooligosaccharides were accomplished: (1) Amylose was successfully blocked with O-benzyl ether groups. 2,3,6-Tri-O-benzyl amylose was prepared for the first time, using benzyl chloride and sodium hydroxide in dimethyl sulfoxide. (2) The perbenzylated amylose was methanolized with hydrogen chloride catalyst to methyl terminal-4-hydroxyl-perbenzylated maltooligosaccharides (I) which were separable at this stage by thin-layer chromatography. (3) The products (I) were methylated to form perbenzylated methyl terminal-4-O-methyl maltooligosaccharides (II). (4) The products (II) collectively were debenzylated either by bromination of the benzyl methylene group, followed by hydrolysis (a new method) or by hydrogenation with Raney nickel at 50° C., 60 p.s.i. Both methods fail with products of D.P. greater than 4 or 5; however, hydrogenolysis was more effective than bromination and hydrolysis at D.P. 5. (5) The deblocked methyl terminal-4-O-methyl-maltooligosaccharides of D.P. 1-4 were separated by both carbon and Sephadex column chromatography.

Studies on unsaturated and sulfur-containing carbohydrates under a grant to Ohio State University have provided high-yielding routes for converting sugars to dimethylthiocarbamate esters, to a new class of furan derivatives, to novel ketoaldehydes, and to derivatives containing 2,3-alkene bonds. Major effort on the reactions of unsaturated sugars under hydrolytic conditions has shown a stepwise series of conversions that are believed to be general for sugars having 1,2- or 2,3-olefinic unsaturation. Structures

proposed in the old literature for a number of transformation products of unsaturated sugars are shown to be incorrect.

Under grant research conducted at Purdue University, nitrogen and sulfur were successfully introduced into the 5 position of sugars by reacting the 3,5-anhydro derivatives of D-glucose and D-xylose with azido, hydrazino, benzylthio, and thiosulfate ions. Phosphorus was introduced at the 1 position via reaction of tetra-O-acetyl-D-glucosyl bromide with sodium diethyl phosphite, but attempts to introduce phosphorus on D-glucose C-5 or C-6 carbon atoms by reacting sodium dibenzyl phosphite or tribenzyl phosphite with 5-O-tosyl or 5-deoxy-5-halogeno derivatives were not successful.

At the University of Arizona, a grant is currently in effect for studies on diepoxide-starch reactions. Suitable procedures for preparation of diepoxides are currently being investigated. Reaction conditions so far explored gave low yields.

At the Plastics Research Institute TNO, Delft, The Netherlands, starch with a high specific surface area (about 100 m^2/g) prepared by methods developed previously in this study was converted to sodium starchates by treatment with sodium methylate in a tetrahydrofuran-methanol mixture (80:20 parts by weight). Under the best conditions the degree of substitution (D.S.) as determined by direct analysis for sodium was only 1.5 out of a possible 3.0. Conversion to the methyl ethers by treatment with methyl iodide under nitrogen gave products with methoxyl D.S. equal to about one-half the original sodium D.S. Apparently about one-half the sodium in the sodium starchates is present as sodium methylate. Considerably lower methoxyl values are obtained if the methylation is done in air instead of under nitrogen. This is apparently due to oxidative reactions of an undetermined nature. Starches with high specific surface areas also were found to adsorb up to 70-80 percent of their weight of butanone, acetonitrile, and tetrahydrofuran. However, rates of adsorption were slower than on charcoal. This work is being conducted under a PL 480 grant.

3. Graft copolymers. In alkaline hydrolysis of a starch-polyacrylonitrile graft copolymer (1:2) (1 graft per 892 AGU; M.W. of grafted polyacrylonitrile (PAN) chains, 286,000) degree of conversion of the intermediate amide to acid was found critical in achieving maximum viscosity. Viscosities of 0.5 percent dispersions of hydrolyzed copolymers were about 10 times greater than most viscous commercial gums, including B-1459. Viscosities in the presence of KCl equalled or exceeded those of CMC and at the 1 percent level were comparable to those of microbial polysaccharide B-1459. Alkaline hydrolysis of a 1:1 copolymer (1 graft per 4,623 AGU; M.W. of grafted PAN, 800,000) gave products characterized by high viscosities, 15,000-26,500 cps. at 1 percent level. In the ceric-ion-initiated grafting of PAN, the number of grafted chains was increased and their average molecular weight reduced by employing aqueous organic solvent systems instead

of water alone. High dilution of starch and acrylonitrile in water also yielded copolymers with more grafted chains and lowered the molecular weight of grafted PAN. The products prepared under high dilution conditions contained about 40 percent PAN, and amount of ungrafted PAN was negligible. There was no correlation between granule size and grafting frequency, indicating that the starch granules were grafted throughout rather than surface grafted.

In research under a PL 480 grant to the Hebrew University, Jerusalem, Israel, ethylene oxide and propylene oxide were grafted by anionic means to cereal starches dissolved in dimethylsulfoxide. Depending on the polyalkylene oxide content of the graft copolymers, graft chain length varied from about 2 to 8 monomer units, and graft chain frequency ranged up to about 1 graft chain per anhydroglucose unit. Copolymers containing from 30 to 35 weight percent grafted polyalkylene oxide were the easiest to purify and were obtained in highest yields (up to 85 weight percent based on the combined weight of starting materials). These products are soluble in water and in methanol but insoluble in acetone. Aqueous dispersions of the products are viscous and sticky. Lauryl methacrylate, 4-vinyl pyridine, methacrylonitrile, and methyl methacrylate were also anionically graft copolymerized with starch. In general, yields were poor and only the 4-vinyl pyridine graft copolymers of starch were water soluble. All other products were insoluble in water and common organic solvents.

4. Thermal reactions of starch. In studies of starch reactions in a dielectric field, conducted under a grant to the University of Pittsburgh, initial experiments with a new 5 Kw. R.F. generator showed a 3.5 percent disappearance of granular starch in 5 minutes. Then, only levoglucosan was detected by GLC in the condensate. Practically all the starch was pyrolyzed in 25 minutes; then four other compounds were detected. Two of these compounds, obtained in yields equal to that of levoglucosan, were tentatively identified as 1,6-anhydro-β-D-glucofuranose and 1,6-anhydro-β-D-galactopyranose. These results were obtained in a simple batch-type reactor. Three types of continuous reactors have been designed and two are being constructed.

At the University of Edinburgh, Edinburgh, Scotland, work on thermal degradation of starches was continued to include a variety of granular cereal starches. Evaluation of the major gaseous products, CO_2, CO, and H_2O, under conditions of high vacuum and temperature ranges of 150-350° C. indicates that apparent differences between starches were small. The thermal stability of amylomaize starch is greatly affected by small amounts of simple inorganic salts. A method of fractionating amylomaize starch was developed which includes treatment of the defatted granules with DMSO and formation and separation of various fractions with butanol and iodine as the respective complexes of these materials. Acid hydrolysis of starch fractions and enzymatic methods of characterizing chain length and amounts of branching

are being studied. A method for determining number average molecular of linear oligomers has been developed using enzyme methods. This work is being conducted under a PL 480 grant.

5. Chemical products from starch. Hypochlorite-oxidized cyanoethylated corn starch (D.S. 0.1) has promising properties for use as a binder for pigment coating of paper. Alkali-hydrolyzed cyanoethylated starch was not satisfactory for this purpose. Preliminary experiments indicated that cationic compounds containing C_{12}-C_{18} fatty radicals are strongly adsorbed by starch and cellulose. Over 50 cationic additives admixed but not directly reacted with starch were screened for cationic efficiency. Imidazoline and trimethyl ammonium chloride derivatives selected from this group for handsheet evaluation required twice the concentration of additive to equal tensile and burst strengths obtained with commercial cationic starches. However, on a cost basis, higher concentrations of cationic additives were still competitive. Clay filler retentions (% ash) on handsheets remained inferior to commercial starch products even at the higher concentrations. Details are being developed for the low-cost synthesis of a cationic reagent, 2,3-epoxy-propyltrimethyl ammonium chloride, for use in reactions with starch.

Water-soluble forms of dialdehyde starch (DAS) are now commercially available. Two of these have been found suitable for use in DAS-protein plywood glue. Collaborative quality testing of DAS-protein glue bond in NU-prepared southern pine plywood panels give scores for the panels ranging from 98 to 100 percent, far surpassing minimum glueline requirements for interior-grade southern pine plywood. Studies on relationship of moisture content of southern pine veneers to satisfactory glue bond of plywood have set guidelines for conducting the American Plywood Association's "A" test for evaluating DAS-protein glue under actual plywood mill operation.

Zinc starch xanthate (ZSX), used as a filler in TiO_2-pigmented natural rubber compositions, resulted in products equivalent to conventional white sidewalls prepared from natural rubber. When used in similar styrene-butadiene rubber (SBR) composition, ZSX gave products equal to SBR reinforced with carbon black and much stronger than SBR containing conventional white fillers. ZSX was found to facilitate incorporation of lignin into SBR. Products had properties superior to those achievable with either filler alone and, for certain formulations, superior to those for SBR reinforced with carbon black. Resorcinol-formaldehyde crosslinked starch incorporated into SBR was found to be highly reinforcing. Resorcinol-resin crosslinked starch gave rubber with higher maximum tensile strength than could be obtained with ZSX. At high loading, the resin crosslinked starch dispersed in SBR better than ZSX and gave smooth, glossy, very strong rubbers, but they were slow in curing. Reaction between starch xanthate and polyacrolein bisulfite in aqueous medium results in a product best characterized as being a thionocarbonate. Preliminary tests show that about 33 percent is the best attained retention of the product thionocarbonate in paper pulp mats.

Under contract research at the Institute of Paper Chemistry, studies on retention of starch xanthates and xanthides by wood pulp show that adsorption of xanthate prior to oxidation to xanthide is not a determining factor in xanthide retention. Performance of starch xanthides formed in situ has been shown to be dependent upon both ionic strength of oxidation system and fiber concentration. However, changes in crosslinking conditions affect these conclusions so that further studies will be directed toward adsorption of starch xanthides.

At the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, commercial hydroxyethyl corn starch of D.S. (degree of substitution) of approximately 0.1 was oxidized with sodium periodate, and the dialdehyde obtained reduced with sodium borohydride. Hydrolysis of the polyalcohol and fractionation yielded erythritol, 2-O-hydroxy-D-glucose, 3-O-hydroxy-D-glucose (trace), and hydroxyethylerythritol. Glucosylerythritol and 2-O-hydroxyethyl glucosylerythritol were also obtained. The results indicate that the commercial product was etherified at the C-2 position of the glucose unit to the extent of about 84 percent of the hydroxyethylation. Most of the remaining ether groups were located at the C-6 position with negligible reaction at C-3.

In other research at this institution, dextrin prepared by heating corn starch and gum karaya in the proportion of 6:4 with 0.07 percent HCl at 153° C. for 8 hours, was exhaustively fractionated with Cetavlon and with barium hydroxide. The results indicate that under the above conditions of dextrinization, copolymerization of the starch and karaya fragments does not take place. Corn starch has also been dextrinized in varying proportions with guar gum, glycine, and gluten at 213° C. without catalyst and at 153° C. in the presence of 0.08 percent HCl. It has been found that glycine, gluten, and guar gum reduce the dextrinization of starch-guar gum mixtures under acid conditions. These investigations were conducted under PL 480 grants.

During the year, studies were completed under a PL 480 grant at the National Institute of Technology, Rio de Janeiro, Brazil. In the final phases of this work, xanthates, thioglycolates, and thiodiglycolates of starch were prepared in efforts to synthesize tertiary sulfonium derivatives for possible use as cationic agents in paper and textile applications. Tetrakis-(hydroxymethyl) phosphonium chloride and 2,4-dichlorobenzyltris (diethylamino) phosphonium chloride were reacted with starch in attempts to prepare quaternary phosphonium derivatives. Other types of modified starches were prepared by reaction of starch with acetylmercaptosuccinic anhydride, chloromethyl-phosphonic dichloride, bis(gamma-chloro beta-hydroxypropyl) phosphite, and hexamethylphosphorus triamide.

6. High-amylose (HA) starch films and chemical derivatives. Films cast from chloroform solutions of high-amylose (HA) starch triacetate containing triethyl citrate plasticizer had clarity, tensile strength, and oxygen barrier properties comparable to those of unmodified HA starch film. Films

of good clarity and strength also were cast from 10-percent aqueous dispersion of HA starch acetate of D.S. 0.2-0.6. Methylated 99-percent-amylose films of D.S. 0.18 to 0.80 were water soluble. Methylated HA starches of D.S. 0.45-0.92 were prepared from Amylon VII (commercial 70-percent HA starch) under conditions of minimum degradation of the polysaccharide. The cold-water-soluble products of D.S. 0.7-0.9 had a diffuse crystalline X-ray pattern, while those of D.S. 0.2-0.4 had a "B" pattern and were hot-water soluble. Films prepared from hot-water dispersions of undegraded acetates of HA starch of D.S. 0.2-0.4 are clear and strong at moderate relative humidities, and retain good oxygen barrier properties. At R.H. below 20 percent, the films require plasticization with 40-percent glycerol to remain flexible.

7. Physical properties of starch. In studies under a contract with the University of Akron, zinc starch xanthate (ZSX) loaded SBR 1500 rubber stocks containing 0-100 parts per 100 parts rubber (phr) were prepared by zinc coprecipitation of starch xanthate with latex (latex masterbatching) and by dry mixing rubber with ball-milled ZSX. When incorporated by latex masterbatching, ZSX reinforced tensile properties of rubber vulcanizates, giving a maximum at about 25 phr loading. Ball-milled ZSX incorporated by dry mixing was nonreinforcing. Microscopic observation showed that maximum tensile strength in starch xanthate latex masterbatches occurred when all starch particles became submicroscopic. Ball-milled ZSX was present in larger particles and hence was nonreinforcing. ZSX in latex masterbatches also had a much higher degree of filler attachment compared to ball-milled dry incorporated ZSX. Rheometer studies on low-substitute ZSX latex masterbatches showed that the xanthate acted as a vulcanization accelerator.

Studies are being carried out under a PL 480 grant at the Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, on the modification of cereal grain starches by a variety of physical and chemical treatments designed to effect changes in their physical properties without destroying the granule structure in order to impart to the starches new and useful paste properties. Treatments performed on samples were: (1) Soaking in dilute acids or salt solutions; (2) heating under different moisture conditions at various temperatures, pressures, and times; (3) freezing under different moisture conditions at various temperatures; and (4) ultrasonic wave penetration of aqueous slurries at different frequencies and intensities. Generally, the various treatments of starches caused a decrease in their viscosity and swelling power, but an increase in their solubility. However, starches modified with sodium phosphate and zinc chloride show an increase in viscosity, swelling power, and solubility; whereas an epichlorohydrin treatment of the starches decreased all three. Of the starches investigated, wheat was the easiest to modify and high-amylose was the most difficult. The other starches were made more susceptible to modification by either defatting or presoaking in distilled water. No detectable difference was found between the X-ray patterns of native and modified starches.

During the year, experimental work on the preparation of starch pastes and exploratory rheological determinations made at paste concentrations of 5-15 percent was initiated under a PL 480 grant to the University of Graz, Graz, Austria. Three main lines of research are being investigated. These are (1) preparation of flow curves from several different types of viscometers, (2) thixotropic decay curves (time dependence), and (3) determination of gel strength and elasticity. The work is in initial phase in that methods for preparing starch pastes having reproducible viscosity characteristics have not been determined. However, some additional equipment which will be acquired should solve this problem. Nevertheless, from some 100 experimental curves, a so-called "diagram of state" for the pastes, in which viscosity is plotted versus the product of the shear rates and the shear stress with time as a parameter, has been prepared.

C. Microbiology and Fermentation

1. ARS Culture Collection. The organism Monascus purpureus is the predominant fungus on certain corn silages that caused cattle to "go off feed" and, in the literature, has been implicated in the death of horses. Large samples of cracked corn and corn meal, each fermented with isolates of this organism, were tested at the University of Illinois for feeding small calves. No evidence of toxicity was observed. Conditions were established for production of $\Delta^{\alpha,\beta}$-butenolide by fermentation with Fusarium nivale. This toxic compound will be administered to calves in the search for the cause of fescue foot symptoms. In 1966, 2,092 cultures were sent from the ARS Culture Collection (1,558 to U. S. investigators and 534 overseas). The Collection was increased by addition of 196 strains to permanent collection and 1,115 to temporary collection, making a total of 33,381 strains in both collections.

Contract research by the American Type Culture Collection revealed that 95 percent of 104 fungi selected for testing, because they failed to withstand lyophilization, may be preserved for at least 18 months when frozen at -196° C. in 10-percent glycerol and stored in or over liquid nitrogen. Most of the others may be preserved if 10 percent DMSO is used as the suspending fluid.

Research of importance to the ARS Culture Collection is in progress at several foreign institutions under PL 480 grants. At the National Institute of Agronomic Research, Madrid, Spain, soil samples are being collected from various parts of Spain and are being screened for presence of streptomycetes elaborating antibiotics active against the plant pathogen Agrobacterium tumefaciens. Ten streptomycete cultures, along with supporting taxonomic data on each, have been received. In addition, extensive cultural and taxonomic data, including electron micrographs, on isolates of Streptomyces species have been received. Since our current need is for cultures rather than descriptive data on random isolates, the original project instructions are being amended to place greater emphasis on the screening of isolates for antibiotic-producing strains. With this revised procedure, a greater

number of cultures may be expected in the future from this source. The 10 strains received to date have been added to our antibiotics culture collection for use in future screening projects.

Soil samples are being collected from various parts of India and screened at the Central Drug Research Institute, Lucknow, India, for presence of streptomycetes elaborating antibiotics active against the plant pathogen Agrobacterium tumefaciens. Seventy-seven streptomycete cultures, along with supporting taxonomic data on each, have been received. Sixty-six of the cultures received have been checked for their ability to produce antibiotics by our methods, and 10 demonstrated activity against Agrobacterium tumefaciens. Twenty-one also were found to produce antifungal antibiotics, inhibiting the test organism Mucor ramannianus. The cultures have been added to our antibiotics culture collection for use in future screening projects.

At the University of Allahabad, Allahabad, India, cultures of Rhizopus arrhizus NRRL 2582, Aspergillus terreus NRRL 1960, Aspergillus niger NRRL 330, and Penicillium chrysogenum NRRL 1760 which produce fumaric and itaconic acids, amylase, and penicillin were investigated for yields of these products. In each instance, 100 spores were isolated at random from lyophil preparations and each isolate was used to make an appropriate fermentation. The yield of the product was determined. One hundred similar isolates were made from a nonlyophilized culture of the same strain and their fermentation products determined. Statistical evaluation of the results of the product yield established that no significant differences existed between the selections from lyophil preparations and the control fermentation. Further studies with microorganisms used to make streptomycin and gluconic acid gave similar results. It can be concluded that lyophilization has no detrimental effect on the capacity of organisms to yield the above fermentation products.

2. Taxonomic investigations. Studies on taxonomy of Mucorales have revealed a (-) strain of Rhizopus microsporus that produces zygospores when mated with the best tempeh-producing strain of R. oligosporus. This (-) strain, identified as R. microsporus on a morphological basis, mated with 22 of the (+) strains, giving from 2 to as many as 1,200 zygospores in a petri-dish mating, depending on the strain of R. oligosporus used. Agar slants of a nutritionally complete medium were inoculated with 202 strains of Rhizopus having small sporangiospores and were incubated at five different temperatures. All except three of the strains both grew and sporulated well at 37° C. All but one strain grew at 40° C., but 19 did not sporulate. Twenty strains did not grow at 45° C. and 50 more either did not sporulate or produced abortive sporangia. Only 71 strains grew at 50° C. and no strains grew at 55° C. Thirty-nine of our 42 strains of R. rhizopodiformis showed a maximum growth temperature of 50° C. However, there was no other close correlation between maximum growth temperature and present species designation.

3. Studies on enzymes. Genetic studies with the original diploid Hansenula holstii resulted in discovery of new homozygous mucoid diploids that produce phosphomannan having viscosity greater than that of the heterozygous parent. Haploid strains which produce even more viscous polymer were in turn obtained from the homozygous diploids. Use of these haploids in continuous fermentation would eliminate troublesome formation of non-mucoid haploids from heterozygous diploids.

Mannitol fermentations have been successfully conducted in 20-liter fermentors. Yields of d-mannitol are 40-50 percent based on the glucose consumed. The organisms used for this fermentation are selected strains of Aspergillus candidus. In the pilot plant, an economical process was devised to recover mannitol from the fermentation broth. This process involves crystallization from aqueous solution under controlled conditions. Preliminary cost calculation for product recovery only, to recover 3 million pounds of mannitol per year from fermentation broth, gave a recovery cost of 10 cents per pound of product. Kinetic studies on the two glucoamylase isozymes of A. awamori NRRL 3112 showed that the first one to appear in the culture has a greater affinity for starch and dextrin than does the second. It was also shown that these isozymes have different rates of glucose formation from starch. Transglucosidase from A. niger NRRL 330 has been isolated free from glucoamylase and has been found to make significant amounts of panose. Glucosyl glycerol has been highly purified and characterized; it contains an α-glucosidic linkage. Synthesis of 2-keto-3-deoxyglucaric acid by use of 6 units of glucaric acid dehydratase is linear over the range of 0 to 50 μ M of glutaric acid.

Microbial spore preparations tested showed considerable metabolic activity on several substrates. A. wentii spores were able to convert soluble starch to glucose; data indicate the presence of a glucoamylase rather than a β-amylase, since no maltose production was detected. Glucose production was influenced by temperature and pH; peak yields were attained in 3 days.

Grant research, completed during the year at the University of Nebraska, showed that the glucoamylase from Rhizopus delemar is similar in molecular weight and mechanism of action to the glucoamylase isozymes isolated from Aspergillus niger, but differs in that it contains significant amounts of glucosamine. The glucoamylases from A. niger appear to be immune to proteolysis by pronase and trypsin. A new glucanosyltransferase which transfers segments of α-1,4-linked glucose units was discovered in enzyme preparations from the bacterium Bacillus subtilis by the novel "oligosaccharide mapping" technique. Conditions were established for the production of glucoamylase with concomitant formation of minimal quantities of α-amylase and glucosyltransferase which will enable direct use of the relatively pure preparations of glucoamylase isozymes.

Under a grant to Kansas State University, substantial progress on the purification of enzymes has been made by refinement of the "stacking" procedure for preparative electrophoretic separations. With horse serum

as an example of a complex protein mixture, good separations of two esterases were obtained using nearly 1 mg. of serum. This is almost 60 times as much material as can be handled by ordinary electrophoresis techniques. One hundred forty milligrams of horse serum have now been fractionated with some success. Distortion of bands remains troublesome in larger scale runs because of convection currents caused by temperature differentials throughout the stacks.

At Iowa State University, also under grant, studies on the action patterns of Streptococcus bovis were broadened to include eight strains of the organism, and five different action patterns were found. A method for noting differences in action pattern was devised. Of the α-amylases so far examined, all but one show multiple attack with characteristic oligosaccharide formation. By use of a model system to follow the action pattern of α-amylase, it was found that the principal point of attack on maltoctaose is at bond 2 or 3 from the reducing end.

Research was initiated during the year under a grant to the University of Arkansas. Results of studies on the mechanism of hydrolysis of cereal starches by enzymes have resulted in a reproducible method for obtaining cyclodextrin transglucosidase from Bacillus macerans. Nearly quantitative transfer of α-cyclodextrin to α-methylglucoside has been obtained. Progress is being made on scaling up the transfer reaction to obtain labelled oligosaccharides for testing with α-amylase from B. subtilis.

Studies establishing structures of peptide subunits in peptidoglycans from several lysine-containing bacterial cell walls are nearly completed. Through a series of sequential enzymic degradations, peptides were obtained from wall preparations. The tetrapeptide sequence N^{α}-(L-ala-D-iso-glutam)-L-lys-D-ala which occurs in apparent precursor uridine nucleotides was shown to be the basal peptide subunit in walls from a variety of organisms. Minor variations include the Micrococcus lysodeikticus peptide which contains a carboxy-terminal glycine residue in place of amide ammonia on the α-carboxyl group of glutamic acid, and Streptococcus pyogenes walls in which 10 percent of the subunits contain hydroxylysine in place of lysine. Considerable strain and species variation was found in crosslinks between wall peptide chains. These bridges between D-ala of one subunit and lysine ε-amino of another are responsible for the three dimensional wall network and its insolubility. The various bridges are: a direct D-ala- ε-N-lys link in M. lysodeikticus, $(gly)_5$ in Staphylococcus aureus, $(L\text{-ala})_3$-L-thn in Micrococcus roseus R 27, $(L\text{-ala})_3$ in another M. roseus strain, $(L\text{-ala})_2$ in S. pyogenes, and N^{α}-L-isoasparaginyl in Streptococcus faecalis. This research is conducted under a PL 480 grant to the University of Liege, Liege, Belgium.

4. Biological insecticides for Japanese beetle. Bacillus popilliae spores, produced to the extent of 10-20 percent on a solid medium, are infective and cause milky disease when injected into larvae. Feeding tests indicate, however, that these spores are not infective when ingested from the soil.

The requirement for specific yeast extract and repression by carbohydrate of sporulation in liquid media containing carbon are identical to phenomena previously observed with solid media. These results indicate that an alternative energy source must be found to achieve good sporulation and that some unique nutrient is limited or out of balance. Heated spores produced on solid media increased infectivity by injection. Optimum temperature for activation appears to be 50-60° C. Spores so activated are almost 2.5 times as infective as unheated spores. Studies on oral infectivity show that larvae must be exposed to at least one billion spores per gram of soil for 21 days to achieve 50-percent incidence of milky disease. The oxygen requirements of healthy and milky larvae were found to be comparable. Hence, the depressed oxygen content of hemolymph during the pre-symptom stage of the disease is not fatal. During the course of milky disease, a protein having a molecular weight of about 300,000 disappears, whereas the concentration of a protein having 30,000 M.W. increases.

In the contract research at Baylor University, electron microscopy showed significant differences in the internal structure of vegetative cells from infected hemolymph and from laboratory cultures. These differences involve membrane organization and, therefore, are significant in sporogenesis. The structure of Bacillus lentimorbus spores is similar to that of Bacillus popilliae spores except that the wall layers are thicker and more dense. The refractile bodies frequently obtained in high yield with some B. popilliae strains consist of ghostlike vegetative forms with empty cytoplasm and accretions of electron-dense material without definite structure. Authentication of the structures formed in liquid medium containing carbon by B. popilliae confirms the first example of sporulation in liquid medium. Grossly distorted structure of most cells and the limited numbers and immature condition of the spores indicate that conditions are far from ideal. This system, however, can now be studied with the knowledge that these structures are not artifacts. Conversely, the concept that refractile bodies are spore forms must be abandoned.

At Michigan State University, enzyme activities of authentic spores, refractile bodies produced in culture media containing β-hydroxybutyrate (BHB), and vegetative cells of strain B-2309A were compared. It was found that vegetative cells contain no catalase, whereas spores from larvae contain a large amount of catalase activity, some of which is heat resistant. Refractile bodies grown in a medium containing barbituric acid likewise displayed partially heat-stable catalase activity. Enolase activity of larvae spores was much lower than that of refractile bodies produced in vitro in media containing BHB. A significant difference has been demonstrated in the metabolism of Bacillus popilliae strains B-2309M, B-2309A, and B-2309S. Strain B-2309M sporulates on agar to a considerable extent, B-2309S to a slight extent, while B-2309A only initiates sporulation and forms refractile bodies. Catalase has been found in strains B-2309M and B-2309A. The enzyme is formed in advance of refractile spores in B-2309M and during refractile body formation in strain B-2309A. This finding

indicates that refractile bodies are a consequence of abortive sporulation. Catalase is not found in young vegetative strains or in nonsporogenic strains at any growth stage. In freshly harvested cells, or extracts of them, the catalase is completely stable to 80° C. for 10 minutes. This work is being conducted under contract.

5. Microbial polysaccharides. Engineering studies on the relationship of oxygen demand to supply in B-1459 Xanthomonas campestris fermentations indicated that oxygen supply is not rate limiting. To investigate retention of vigor by B-1459 organism, fresh medium was added to an aliquot of a 48-hour fermented broth left from a preceding run. This back inoculation cycle was repeated 10 times over a period of 25 days. Results showed that culture retained most of its vigor and, therefore, that decreased vigor should not be a factor in continuous fermentation. Maintenance of stock cultures of B-1459 by a new procedure has overcome culture instability experienced in past fermentations. Polysaccharide productivity of the culture was good and viscosities 6,000 cp. were obtained in 48-hour fermentations. In a continuing effort to have a uniform polymer-producing microorganism, colonies of B-1459 were isolated and tested for polysaccharide production. An isolate, B-1459A, showed good polymer-producing capability.

A new modification of the carbazole analysis was developed for the measurement and identification of specific uronic acids in the presence of other uronic acids and/or neutral sugars. Structure of polysaccharide B-1973 has been established. It was found that the D-mannuronic acid moiety has the pyranoside form and that both the D-glucose and D-galactose moieties are glycosidically bonded at C_4. Also defined was the sequence of components in the component aldouronic acids. A vigorous producing culture of polysaccharide B-1828 has been developed and maintained; properties suitable for preliminary characterization of the product polysaccharide have been determined; and additional data pertinent to chemical structure were obtained. By an improved isolation procedure, a good supply of n-acetyl glucosamine polymer was obtained from growth medium of an unidentified yeast strain. Structural work on this polymer has revealed a neutralization equivalent of 740, indicating one acidic function per trisaccharide unit; acetyl of the acetamido function is the only identifiable acyl group after acid hydrolysis; acidity is not due to esterified sulfate or phosphate; polysaccharide is not oxidized by sodium periodate and thus is probably linked 1,3 throughout.

In studies on biphasic fermentation, under a grant to Cornell University, it was found that addition of Vitamin K partially reverses the growth of yeast by paraffin hydrocarbons. By coating either the microorganism or the solid surface with positively charged colloids, such as colloidal aluminum oxide, very strong adherence of the cells to a surface can be attained. This technique suggests possibilities for an improved fermentation method, cell coagulation, and increased resistance to lysis.

Under grant research at the University of Wisconsin involving studies of the fine structure of microbial polysaccharides, conditions were established for dispersion in organic solvents and carbanilation of polysaccharide B-1973. Selective hydrolysis of O-acetyl groups in the presence of O-carbanilyl groups has been investigated using the model compound 2,3-di-O-acetyl-4,6-di-O-carbanilyl-α-D-glucopyranoside.

6. Plant antibiotics. In tests on nine antibiotic preparations against 12 plant diseases and root nematode infestation, two were active against early blight of tomatoes, one against late blight of tomatoes, two against mildew of green beans, six against rust of beans, two against black spot of roses and three were effective in controlling certain soil fungi causing damping off diseases of seedlings; none was active in control of root knot nematode. These are in addition to the 10 stable non-polyenic antibiotics previously found to have promise for controlling economically important plant diseases. A new broad-spectrum antibiotic produced by several Alternaria species has been discovered; it has both antifungal and antibacterial activity.

7. Microbiological processes and products. Research on genetic modification of fermentation showed that diploids obtained by crossing haploid segregants of Hansenula holstii diploid Y-2448 produce phosphomannans differing qualitatively and quantitatively from those produced by the parents. The new diploids displayed poor sporulation so that further study of the genetic factors determining the kind and amount of phosphomannan produced could not proceed effectively. Ultraviolet-induced nutritional mutants of the parent strains were studied, but again sporulation was blocked in the constructed diploids. Attempts to derive breeding stocks which produce the normal complement of four spores per ascus have been unsuccessful. Recovery of all meiotic products from H. holstii asci containing only two spores indicates that secondary causes may be limiting formation of the expected four spores. The intensity of agglutination present in progeny from matings between moderately strong agglutinating H. wingei ranged from very strong through barely perceptible. Similar patterns appear in progeny of both mating types. Results suggest that agglutination is regulated by several factors which are not linked to the mating locus.

In the Pioneering Laboratory for Microbiological Chemistry, investigation of the genetic defect of Rhodospirillum rubrum that interferes with mutant identification on the basis of colonial morphology revealed that the lesion may result from a deficiency in d-alanine production necessary to form crosslinking wall precursors in the peptidoglycan layer. Further studies of ornithine lipid of R. rubrum indicated three classes of hydrolysis products: amino acid, polyols, and fatty acids and aldehydes. Fatty ester distribution on the polyols resembled the phospholipid fraction of R. rubrum, but the fatty acyl distribution resembled the bound lipids. Investigations on macromolecules responsible for agglutination of certain yeast strains

have revealed the disulfide bond structure is an important factor in the activity of the sex-specific agglutinin of type 5 Hansenula wingei. Fractionation (on Sephadex G-100) of the agglutinin in which the disulfide linkages had been broken by reduction yielded components having, respectively, sedimentation rates of 2S and 13S. Agglutinative activity was restored to a mixture of these components, but not to either singly, by dialysis in alkaline buffer. Therefore the structure of each component must be uniquely involved in the mechanism of agglutination.

In other work in the Pioneering Laboratory, differing yeast species are being surveyed to determine the prevalence of zymonic acid formation and the relationship to thiamine deficiency. New routes to the synthesis of 2-oxo-4,5 dihydroxyvaleric acid, a probable intermediate in the oxidation of L-arabinose by Pseudomonas saccharophila, are under investigation.

Under a contract to the University of Minnesota, an effort to determine whether the genetic elements of sporulation in Bacillus cereus T may be contained in an episome has resulted in finding a satellite DNA which appeared briefly in B. cereus T upon germination and before the first cell division. It could represent a genetic fragment in spores that are involved in control of sporulation and germination. Further work with DNA from three different phages and a variety of strains of B. cereus shows that B. cereus cells do not take in exogenous DNA. This species is, therefore, not suitable for study of genetic control of sporulation. It was found that in mixed culture with a Rhodotorula yeast, up to 18 percent of B. popilliae cells develop refractile bodies after 25 days. Addition of the yeast during the first 24 hours of culture was most effective; addition after 48 hours was practically ineffective.

At the University of Newcastle upon Tyne, Newcastle upon Tyne, England, research under a PL 480 grant has been completed and structural details of the teichoic acid (TA) extracted from Streptomyces griseus have been worked out. As has been found in TA from other actinomycetes, alanyl ester is absent although small amounts of ester-bound acetic and succinic acids were found. Various degradation techniques gave phosphodiester and monoester fragments which were isolated and characterized. The TA from S. griseus is unusual in that glycerol and ribitol phosphates occur in the same polymer. About eight ribitol units are linked as 1,5' phosphodiesters. Appended to some of the ribitol 3-positions are about 5 L-α-glycerophosphoryl side chains randomly distributed. Ribitol triphosphate and its degradation product, anhydro-ribitol diphosphate, were found, further indicating that some of the ribitol units bear more than two phosphate groups. No glycerol phosphate is present on the glycol end of the chain. β-D-glucosyl units are attached to the 2-positions of adjacent ribitol phosphates. Since no degradation product containing glucosylated ribitol phosphate linked to ribitol bearing a glycerophosphoryl has been found, two distinct TA's may be present, bearing glucosyl and glycerophosphoryl, respectively. The small amount of bound peptide present contains components characteristic of cell wall mucopeptide.

Last year it was noted that work had been completed under the PL 480 grant to the University of Milan, Milan, Italy. The final report, which was subsequently received, showed that in the strain of Acetobacter suboxydans tested, the two ketogenic enzyme systems which yield 2-ketogluconic acid and 5-ketogluconic acid were constitutive. The amount of these enzymes depended upon the physiological condition of the cell. These results mean that the enzymes for either product cannot be uniquely induced by specific substrates or other components in the medium. At the same time, the activity pattern of the enzyme systems make it impossible to choose cultural conditions in which one product is obtained to the complete exclusion of the other. Thus, it is concluded that formation of 5-ketogluconic acid as the sole fermentation product will require a strain which inherently lacks the capacity to form 2-ketogluconic acid. Such a strain will have to be obtained by selection of unique varients or induction of such varients by mutation.

8. Fermentation acids. In research at the University of Lodz, Lodz, Poland, irradiation of Aspergillus terreus by ultraviolet light or by gamma rays has yielded several strains which produce about three times as much itatartaric acid as does the parent strain. In surface fermentation yields as high as 6.5 g. per liter of itataric acid have been produced in 23 days in medium containing 17-19 percent glucose. Yields are not as high in submerged fermentations. Itatartaric acid and β-hydroxyparaconic acid are interconvertible at pH 2.0 and the equilibrium mixture is nearly 1:1 after 19 days at 35° C. Presumably the β-hydroxyparaconic acid found in A. terreus fermentations arises from itatartaric acid. None of the strains studied utilize itatartaric or β-hydroxyparaconic acids while all but one strain utilizes all the other organic acids formed during the fermentation.

Research on fermentation acids is in progress under PL 480 grants at several foreign institutions. At the University of Tokyo, Tokyo, Japan, factors affecting mevalonic acid production by the yeast Pichia membranefaciens IAM 4032 were investigated. The best medium contained glucose, yeast extract, potassium phosphate, ammonium chloride, magnesium sulfate, and calcium carbonate. Glucose was the only suitable carbon source for mevalonic acid production with 0.1 percent yeast extract and 0.2 percent phosphate also required for optimum yields; other components were less essential. The highest yield was 150 µg per ml. (130 µg average) in 4-5 day fermentations. An isoprenoid produced by Trichothecium roseum Link IFO 5772 was also investigated. This compound was extracted with acetone from mycelia grown 96 hours in aerated fermentors using a glucose, peptone, yeast extract medium. The compound has strong antiviral activity against Newcastle Disease Virus strain Miyadera in cell culture and against Candida albicans. It has very low toxicity to mice. Infrared and ultraviolet spectra of the crystalline compound showed it to be tricothecin. Although known as a fermentation product, its antiviral properties were unknown.

In other research at the University of Tokyo, screening of freshly isolated organisms from soil has turned up additional organisms that appear to produce tartaric acid. Strains from three different genera have been found which produce significant amounts of the acid. Analytical methods for determination of tartaric acid have been refined. Both polarographic and photometric methods have been found to be satisfactory. Artificial mutants of promising strains have been prepared by UV irradiation and are being assessed.

At the National Sugar Institute, Kanpur, India, aldobi- and aldotriuronic acids are being isolated from plant gums in amounts sufficient for use as reference standards in NU research on microbial polysaccharides produced from cereal grains. Nearly completed is the series of aldobiuronic acid (isolated as the Ba salt): X-O-(β-D-glucopyranosyl uronic acid)-D-galactose (in which X is 6, 4, or 2). Also obtained has been X-O-(β-D-galactopyranosyl uronic acid)-hexose (in which X is 4 or 2 and the respective hexose is D-galactose or L-rhamnose). The remaining isolates to date are two triuronic acids containing 2-O-(-glucopyranosyl uronic acid) and 2-O-(-galactopyranosyl uronic acid), respectively. For each product, specific conditions have been established for the critical steps of partial hydrolysis of the purified gum by sulfuric acid, fractionation of the hydrolyzate on exchange resin, and purification by resin or paper chromatography.

D. Technology--Process and Product Development

1. Cereal xanthides and xanthates. The new 32-inch experimental papermaking facility has been calibrated and is considered fully operational. A wide range of paper products ranging from tissue to linerboard have been produced. A system for continually introducing additives to the pulp furnish has been developed, installed, and integrated into the total papermaking operation. Crosslinked xanthate has been continuously applied to kraft bag paper. Several NU-prepared cationic starches and flours applied at wet end provided strength improvement in paper handsheets similar to that obtained with commercial cationic starches, but starch graft copolymers did not prove as efficient as the commercial material. Performance of acid-modified wheat flour (AMF) as a surface size was good.

Investigations of redistribution of xanthate groups in starch xanthates were completed. This work was based on study of model compounds--the pure, isomeric 2-, 3-, and 6-O-(sodium xanthates) of methyl-α-D-glucopyranoside. When dissolved in 18 percent sodium hydroxide, the 2- and 3-isomers were converted to the 6-isomer which remained unaffected. The mechanism for this rearrangement is believed to depend on orthoester intermediates involving neighboring hydroxyl groups. Studies with amylose and model xanthides demonstrated that thionocarbonates are readily formed as decomposition products of xanthide. These studies support the proposal that such a thionocarbonate linkage might chemically bond starch to cellulose when starch xanthide is incorporated as a wet-end additive. When paper made with starch xanthide was treated with water at 80° C. for 90 minutes, all of the xanthide decomposed, while the high wet strength of the paper was unaffected.

CS_2 and elemental sulfur were formed. Infrared analysis of treated paper showed an absorption band characteristic for a thionocarbonate group and no absorption for a xanthide group. When the hot-water-treated paper was subjected to sodium carbonate solution (pH sufficient to destroy thionocarbonate), the resulting paper showed no wet strength.

2. Graft copolymers. Under a research contract, a 100-pound lot of starch-acrylamide (AA) graft copolymer has been prepared, characterized, and shipped to NU by Stanford Research Institute. Content of grafted AA was 18.3 percent, $\bar{M}_w$ for the grafted chain was 32,000, and grafting frequency was 1 per 880 AGU. At 25° C., aqueous pastes of this copolymer and the parent unmodified wheat starch had comparable viscosities at pH 5.5 and 10.5. Two other lots of AA grafted copolymers shipped to NU contained 7 and 18 percent grafted AA. Weight average molecular weight and grafting frequency in 7 percent product was 109,500 and 8,840, respectively. Corresponding values for the other copolymer was 222,000 and 6,100. Cost estimates, based on annual production of 5 million pounds of AA graft copolymers, give estimated selling prices of 24 and 28 cents per pound, respectively, for grafted copolymers with 7 and 18 percent grafted AA.

3. Starch derivatives for protective coatings. At Archer Daniels Midland Company, under a research contract, alkyd resins containing 40 to 70 percent oil (linseed or soybean) were prepared by reacting the alcoholysis products of glycol glycosides, glycerol, and soybean or linseed oils with various anhydrides of dibasic acids. Mixtures of succinic and maleic anhydrides gave the most promising results. Acceptable color was achieved by use of decolorizing charcoal during cook and by controlled addition of glycoside to cook. Workable viscosities were obtained by increasing excess hydroxyl content to 20 percent with glycerol. Four samples of alkyd resins (60-70 percent oil) were found to have film properties comparable to those of standard resins with regard to drying time, Sward hardness, and alkali resistance.

Under contract research at Battelle Memorial Institute, exterior and interior latex paint formulations were made with four starch derivatives as viscosity stabilizers. After 3 months' exposure, no major changes were noted in panels coated with the exterior formulations. Interior paint exposure after 1 month showed no major differences between control paint containing methyl cellulose as protective colloid and those made with the various starch derivatives. However, longer interior exposures to north light produced an increase in yellowing. Freeze-thaw stability results on the latex paints indicated that potassium carboxymethyl starch is as effective as methyl cellulose as a protective colloid.

4. Cyanoethylated starch. Corn starch absorbs sodium hydroxide from an aqueous solution according to a Freundlich isotherm. Studies over a temperature range of 4 to 55° C. showed that the Freundlich exponent varied little with temperature, and the coefficient decreased slowly with increasing temperature. Two different measurements of swelling have confirmed

that swelling is a function of concentration of alkali in starch after absorption, as well as temperature. The NU process for acid modification of soft white winter wheat flour was tested successfully in large-scale equipment of a flour mill. Product from one of the tests was successfully applied to paper as a surface size on the 32" paper machine. It required 50 times more acid to achieve the same rate of starch modification in an aqueous slurry as in dry-process modification with gaseous HCl.

At Western Michigan University, contract work on the use of cyanoethylated modified corn starches in paper processes is completed and evaluation is in process. The machine runnability of the cyanoethylated starches is comparable to that of the control commercial starches. Film properties of cyanoethylated starches are excellent with somewhat higher Hercules viscosity being compensated for by the higher thixotropy and leveling index of these starch coatings. All coated paper properties after super-calendering are comparable to those achieved with the various control starches with the exception that acid-modified cyanoethylated starch imparted much higher surface strength (pick resistance) and lower ink absorption. In the size-press study, acid-modified cyanoethylated starch was found superior to a commercial oxidized potato starch. In comparison to a hydroxyethylated starch, acid-modified cyanoethylated starch yielded papers of equivalent properties except for tensile strengths, fold, and smoothness.

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AREA NO. 2: CORN UTILIZATION - FOOD

Problem. Utilization of corn in products for human consumption is an outlet of great economic importance. In 1964, U. S. per capita consumption of meal, cereal food, sirup, sugar, and starch obtained from corn totaled 28.3 pounds. If corn used for production of alcoholic beverages is included, this figure would be about 25 percent greater. In processing corn for food and beverage uses, corn oil is obtained as a valuable byproduct. Since 1964, annual production of corn oil has exceeded 400 million pounds. Except for foots and refining losses, all of this oil is consumed in food products, principally margarine and salad and cooking oils.

The need for research is encountered primarily in dry milling of corn where the yield and quality of fractions are important both economically and in terms of consumer acceptance and nutritive value of final products. Improvements are needed in milling techniques, especially for old and artificially dried corn, if optimum results are to be obtained. More information is needed on the composition of corn and corn fractions in order to identify and minimize losses of nutritionally important components that may occur during processing. Such investigations should result in cheaper and more nutritious products and therefore contribute to increased utilization.

In addition, these studies provide a foundation for research on composition, processing, and utilization of new strains of corn now being developed that have significantly higher nutritional value than does ordinary corn. Success in this development could make corn the world's No. 1 food grain. U. S. corn production, which is now 4 billion bushels annually, could be readily expanded to neutralize the present 1-billion-bushel annual world shortage of cereal foods. Since this new corn differs physically from ordinary corn, much effort will be needed to develop suitable milling methods and to provide the knowledge necessary for development of suitable food products to meet dietary needs of world populations.

It has recently been discovered that certain oilseeds and cereal grains, including corn, are subject to infection by molds that can produce toxic products. To provide safe food products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from corn.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of corn in food.

The Federal scientific effort for research on food utilization of corn totals 7.4 scientist man-years. Of this number, 2.2 are devoted to chemical composition and physical properties; 2.6 to color, texture and other quality factors; 1.4 to microbiology and toxicology; and 1.2 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (1.5 scientist man-years) is concerned with lipids and proteins of the corn kernel. A grant has been made to the University of Missouri, Columbia, Missouri, for studies on the interaction of phytin with proteins in processing corn (.7 scientist man-year).

Research at Peoria, Illinois, on color, texture and other quality factors (2.6 scientist man-years) involves study of the reactions of corn sugars with amino compounds.

Research at Peoria, Illinois, on microbiology and toxicology (1.2 scientist man-years) is devoted to studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.2 scientist man-year) is allocated to research on food uses of corn.

Research conducted at Peoria, Illinois, on technology--process and product development (.9 scientist man-year) comprises investigations on corn dry milling. A grant is in effect at Pennsylvania State University, University Park, Pennsylvania, for basic studies on the mechanical and viscoelastic properties of shelled corn as related to the corn dry-milling process (.3 scientist man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.6 scientist man-years is devoted to research on food uses of corn.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Corn proteins, lipids and nonprotein nitrogen components. Compositional studies on corn are relevant both to food and feed utilization. Results are reported under "Corn Utilization - Feed," subheading A.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are relevant both to food and feed utilization of corn. Results are reported under "Corn Utilization - Feed," subheading B-1.

C. Technology--Process and Product Development

1. Corn dry milling. Tests on the response to cold tempering and degermination of corn initially containing 21, 17, and 13 percent moisture indicate that milling of the 13-percent-moisture corn was most affected by the tempering conditions. Increasing temper time gave some improvement in the performance of the 13-percent-moisture corn. The 21-percent-moisture corn gave more flaking grits of lower oil content and more recoverable oil. Milling results with this corn were more consistent than with the lower moisture corns. Additional tests on stress-crack formation during tempering of corn indicate that degree of drying and pretempering influence the extent of stress-crack formation. Work on water flotation of milled corn fractions has shown that good separation of free germ and of high germ fragments from endosperm can be obtained using feed of a particle size range as broad as -4 to +16 U. S. sieve.

2. Mechanical and viscoelastic properties of corn kernels. Under a research grant to Pennsylvania State University, new and better data are being obtained on swelling stresses occurring in the corn kernel during moisture absorption. For a corn initially containing 12 percent moisture, these stresses were of the order of several thousand pounds per square inch. The swelling stresses dropped sharply as the initial equilibrium moisture content increased. Rheological studies showed dependence of deformation characteristics on moisture content of corn kernels. The horny endosperm was the major contributor to the mechanical properties of the corn.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Technology--Process and Product Development

Black, L. T., Spyres, G. G., and Brekke, O. L. 1967. Determination of oil contents of dry-milled corn fractions by gas-liquid chromatography. Cereal Chem. 44(2), pp. 152-159.

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AREA NO. 3: CORN UTILIZATION - FEED

Problem. About 75 percent of the U. S. annual production of corn is used as animal feed. Corn is fed to animals in various forms including ear corn, shelled corn, cracked or ground corn and, in certain mixed feeds, corn gluten and other milling fractions. Because of the extremely large volume of this outlet, even small improvements in quality or processing efficiency are economically important to the feed industry and to the farmer.

The components responsible for certain nutritional effects attributed to corn, such as growth stimulation and improved feed utilization efficiency, have not been satisfactorily identified, nor are processing steps available that take these components into account. More information is needed generally on the nutritionally important components of corn and on the changes that occur in them during processing. Besides their activity as Vitamin A precursors, carotenes contribute desirable color to milk and the body fat of cattle. Xanthophyll pigments similarly impart yellow color to egg yolks and to the skin of broilers and fryers. Adequate information on these pigments and on their fate during processing is also needed to insure maintenance and improvement of quality.

Compositional and related processing research is required on several new strains of corn that are expected to become commercially important. These strains include (1) those expected to lead to corn capable of providing a balanced source of amino acids, and (2) those that contain increased amounts of xanthophyll and other carotenoid pigments and therefore would improve the competitive position of U. S. corn in world markets.

Another important direction for research is the fermentative conversion of corn grain and corn sugar to nutritionally important feed additives. The value of corn-based media for production of vitamins, β-carotene, and antibiotics is well known. However, possibilities for additional important developments are virtually unlimited and should be investigated on a continuing basis. Corn steep liquor is frequently used as a supplement in fermentation media and may also be added to feedstuffs. More information is needed on corn steep liquor to identify the factors responsible for its stimulating effects on growth.

It has recently been discovered that certain oilseeds and cereal grains, including corn, are subject to infection by molds that can produce toxic products. To provide safe feed products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from corn.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of corn in feed.

The Federal scientific effort for research on utilization of corn in feeds totals 2.9 scientist man-years, of which 1.5 are devoted to chemical composition and physical properties and 1.4 to microbiology and toxicology.

Research at Peoria, Illinois, on chemical composition and physical properties (1.5 scientist man-years) involves study of lipids and proteins of corn kernels.

Research at Peoria, Illinois, on microbiology and toxicology (1.2 scientist man-years) is concerned with studies on the production of mycotoxins by Aspergillus flavus and related molds. A research contract (.2 scientist man-year*) is in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites. During the reporting period, research was completed at A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene and with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains (4 years, 1966-1970). Effort on this project is prorated among corn, wheat, and sorghum.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.6 scientist man-years is devoted to research on industrial and feed uses of corn.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen components. In research on corn proteins, improved procedures are being developed for isolation of the various classes of corn

*Work covers more than one commodity; only effort allocated to corn is included in total.

proteins by extraction with different solvents. Dialysis of 0.5 M sodium chloride extracts of whole corn against distilled water precipitated globulins and left albumins in aqueous solution. The globulins accounted for 8-12 percent of the corn nitrogen; the albumins represented 7-10 percent, depending on extraction and dialysis conditions. Differences in starch gel electrophoresis patterns were noted between albumins and globulins, although amino acid differences between these fractions were not great. Fractionation of albumins on Sephadex demonstrated that these proteins differ in molecular weight, possibly due to aggregation or disulfide crosslinks. After removal of salt-soluble and 70-percent alcohol-soluble proteins, residual protein was extracted with 0.1 N NaOH to yield a glutelin fraction. This protein was high in molecular weight and did not migrate electrophoretically on starch gel. Further studies on this protein established that glutelin is heterogeneous and may be a mixture of proteins. It contains several components, some of low mobility like that of zein, others of high mobility like that of globulins.

2. Lipids in hybrid corn kernels. Research on corn lipids has resulted in the development of a procedure, based on gas chromatography (GLC), for determining small amounts of triglycerides in corn and corn fractions. Three commercial dent corns were hand-dissected into pericarp plus tip cap (7% of kernel), endosperm (85%), and germ (8%). Crude fat was extracted with n-hexane. Hexane-soluble material in whole corn ranged from 3.7 to 4.5 percent; in pericarp plus tip cap, from 5.1 to 6.5 percent; in endosperm, from 1.9 to 2.0 percent; and in germ, from 27.3 to 32.9 percent. Separation of the lipids by thin-layer chromatography indicated that the various fractions had similar overall class lipid compositions. The largest single fraction was composed of triglycerides. Smaller amounts of hydrocarbons, fatty acids, monoglycerides, diglycerides, sterols, and phospholipids were also present. Triglycerides were separated by GLC on a 3-percent JXR column. The three fractions had similar triglyceride compositions. Average triglyceride composition was C_{50}, 4 percent; C_{52}, 30 percent; C_{54}, 64 percent; and C_{56}, 2 percent. (Designation refers to number of carbon atoms in the fatty acid moiety of the triglyceride.)

B. Microbiology and Toxicology

1. Aflatoxin investigations. The survey for the presence of aflatoxin in oats, corn, and soybeans has now been completed. Two out of 304 oat samples assayed by a modified procedure had 3-6 p.p.b. aflatoxin B_1. Of the 866 samples of soybeans collected, six had 3-6 p.p.b. aflatoxin and one had 7-19 p.p.b. Five positive soybean samples were combined and the presence of aflatoxin confirmed by the duckling test. Aflatoxin was detected at levels of 3-6 p.p.b. in 11 samples and 7-19 p.p.b. in 24 samples of the 1,311 corn samples tested. Positive samples were in all grades of corn except grade 1, but only a total of five samples were positive in grades 2, 3, and 4. The presence of aflatoxin was confirmed biologically in about one-third of the corn samples. One strain of Scopulariopsis brevicaulis

was found to completely and irreversibly remove aflatoxin B_1 from solution. Strains of two Aspergillus species capable of partial removal of aflatoxin were also discovered. Flavobacterium aurantiacum was shown to remove aflatoxin G_1 as well as B_1 from solution. Indeed, cells completely saturated with B_1 readily took up G_1, showing that different metabolic sites are involved. Nocardia species have been shown to actively remove aflatoxins from solution. Engineering studies showed that to maintain good aflatoxin production on solid-rice substrate, no culture beyond third generation from lyophil should be used.

In research under a contract to the University of South Dakota, initial results have revealed a new aflatoxin strain that is nearly as potent as NU's best selection. Survey of 107 Aspergillus strains has picked up a strain of A. parasiticus (NRRL 465) which, when grown on soybeans, is the strongest aflatoxin-producing strain yet encountered. It forms many more times the aflatoxin produced by A. flavus (NRRL 2999) under similar conditions. The A. candidus and A. versicolor groups are Aspergilli fairly regularly encountered in cereals. None of the later strains tested showed toxicity. A strain of Aspergillus clavatus was found to cause reduced growth in chick-feeding tests; however, this strain does not produce aflatoxin. Two or three mildly toxic strains were encountered in A. sydowi and A. versicolor groups, including one strain recently isolated from wheat flour. Microscopic examination of livers of the chicks fed material from these cultures showed liver changes.

2. Beta-carotene synthesis. Under a PL 480 grant to the Agricultural University, Poznan, Poland, production of beta-carotene by mated cultures of Blakeslea trispora is being studied. Particular emphasis is being placed on the factors in spent mycelium, from a previous fermentation, that greatly enhance carotene yields. Initial work was concerned with replacing medium components previously found to be optimal for production with substances available in Poland. Pharmamedia was replaced by brewers yeast and ground whole soy; Deobase by Polish-made kerosene "KB"; the detergent Triton X-100 by "Alfenol"; citrus molasses by beta-ionone. Yields almost equivalent to those obtained with the original medium were attained (100 mg./100 ml. medium). Spent mycelium added in place of citrus molasses or beta-ionone stimulated carotene synthesis but not to the same extent as did the replaced substances.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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*Research supported by PL 480 funds.

AREA NO. 4: WHEAT UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Although the principal use of wheat is as food, over 200 million pounds of wheat starch and flour was consumed by industry in 1965. Loss of this market would detract from the economic value of wheat as a crop. As a food grain, wheat commands a price that is generally unfavorable to its utilization as an industrial raw material. However, in certain areas, notably the Pacific Northwest where corn is not grown, wheat is cheaper than either corn or sorghum. Furthermore, new high-yielding strains of wheat especially suited for this area are being developed. There are many paper mills in this area, and the need for technology to use starch and flour produced there has materialized. Much of the starch now used comes from imported tapioca.

Other possibilities for economic and noncompetitive industrial outlets for wheat are based on use of whole ground wheat and millfeeds, including wheat bran, and on exploitation of the properties of wheat gluten protein, which has unique properties not possessed by other cereal proteins.

Not only paper and paper products, but also coatings, adhesives, thickeners, and plastics offer excellent opportunities for industrial products derived from wheat. A more detailed discussion of industrial outlets for cereal starches and flours is given in Area No. 1, Corn Utilization - Industrial Products.

To achieve the objective, research is needed to learn how wheat flour, starch, and milling fractions can best be modified to provide new and improved properties such as water resistance, dispersibility, paste viscosity, tack, and adhesive bond strength. The possibilities of achieving some of these improvements by modification of the gluten component of flour should be investigated. Conditions must be established for optimum use of industrially promising products now under study such as acid- and enzyme-modified flours and xanthated bran and millfeeds. Basic research should provide leads to other products and processes for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of wheat, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of wheat totals 52.3 scientist man-years. Of this number, 5.4 are devoted to chemical composition, physical properties and structure; 18.5 to chemical and physical investigations to improve products; 16.5 to microbiology and fermentation; and 11.9 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (4.0 scientist man-years) involves study of wheat flour, starch, and the component proteins of wheat gluten. Research on wheat starch is integrated with that on corn and sorghum starches. The work on wheat includes study of the microscopic and ultrastructure of wheat grains and flours and of changes induced by various treatments. During the year, studies on the rheological properties of starch were initiated. Contract research at the Purdue Research Foundation, Lafayette, Indiana, for studies on alkaline desulfurization of wheat gluten proteins has been completed. Grants (1.4 scientist man-years) are in effect at Marquette University, Milwaukee, Wisconsin, for basic studies on intermediates involved in forming glycoprotein linkages; at Iowa State University, Ames, Iowa, for basic research* on heat, mass, and momentum transport of cereal starches and flours; at Purdue Research Foundation, Lafayette, Indiana, for research* on the effects of disulfide bond cleavage on the structure of corn and wheat endosperm proteins; and at the State University of New York, Syracuse, New York, for investigations of starch fine structure.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (11.9 scientist man-years) includes study of the chemical reactions of wheat starch, flour, protein and milling fractions with the objective of discovering new chemical products and processes having potential for industrial use. Research on wheat starch is integrated with that on corn starch. During the year, studies on starch-based plastifoams were completed and research on related noncellular plastics was initiated. Research contracts (3.2 scientist man-years) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies* on the reaction of acetylene with methyl glucoside; with the University of Akron, Akron, Ohio, for evaluation of starch and starch derivatives as reinforcing agents for natural and synthetic rubber; with Southern Illinois University, Carbondale, Illinois, for investigations* on synthesis of maltooligosaccharides; with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation* of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper; with General Mills, Central Research Laboratories, Minneapolis, Minnesota, for studies on the development of cereal proteins having utility as flotation and flocculating agents; and with IIT Research Institute, Chicago, Illinois, on preparation, characterization, and chemical modification of polypeptides derived from wheat gluten. Contract research was completed by The Johns

*Work covers more than one commodity; only effort allocated to wheat is included in total.

Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media, and with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers. Grants (3.4 scientist man-years*) are in effect at Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; at Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; at Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur; at the University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; at the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides; at Southern Illinois University, Carbondale, Illinois, for studies on the alcoholysis of carbohydrate esters; and at the University of Chicago, Chicago, Illinois, for studies of reactions and transformations of serine in proteins and peptides.

Research on microbiology and fermentation conducted at Peoria, Illinois, (13.1 scientist man-years) includes studies on the use of microorganisms to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. This research is integrated with similar studies based on corn. A large collection of pure cultures of industrially and agriculturally important microorganisms is maintained. The Pioneering Laboratory for Microbiological Chemistry conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (.9 scientist man-year*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation; at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective; and at Michigan State University, East Lansing, Michigan, for investigation of the biochemical properties of variant cultures of Bacillus popilliae. Contract research at the Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms has been completed. Grants (2.5 scientist man-years*) are in effect at Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; at Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; at Iowa State University, Ames, Iowa,

*Work covers more than one commodity; only effort allocated to wheat is included in total.

for investigation on bacterial amylases and their action patterns; at the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; at the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch; at the University of Nebraska, Lincoln, Nebraska, for structural studies of fungal glucohydrolases; at Baylor University, Houston, Texas, for cytology of ascospore formation in yeasts; at the University of Minnesota, Minneapolis, Minnesota, for studies of cellular differentiation and physiology of selected molds; at East Texas State University, Commerce, Texas, for determinations of branching in polysaccharides; and at Indiana State University, Terre Haute, Indiana, for surveys of gum-producing microorganisms. During the year, grant research was completed by Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes.

Research conducted at Peoria, Illinois, on technology--process and product development (6.4 scientist man-years) is concerned with detailed study and evaluation of wheat-derived products having definite potential for industrial utilization and of processes for making them. Also, studies are conducted on modified techniques for milling and fractionating wheat to obtain improved materials for industrial and other purposes. During the year, milling research was redirected to emphasize discovery and evaluation of new and unconventional approaches. Research involving chemical modification of wheat starch is integrated with that on corn starch. Research contracts (5.5 scientist man-years) are in effect with Stanford Research Institute, Menlo Park, California, for process development* of selected starch graft copolymers; with Western Michigan University, Kalamazoo, Michigan, for evaluation* of modified cyanoethylated starches for applications in paper; with Battelle Memorial Institute, Columbus, Ohio, for development of optimal processes for incorporating wheat-derived xanthides into paper products, and for studies* on starch derivatives for use as colloids in water-emulsion paints; with Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations* on use of starch glycosides in coatings and plastics; and with the Brown Company, Berlin, New Hampshire, for evaluating acid-modified flour as a paper size. During the year, contract research on starch and other cereal grain xanthides was completed by Battelle Memorial Institute, Columbus, Ohio.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds.** Research on chemical composition and physical properties involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968);

*Work covers more than one commodity; only effort allocated to wheat is included in total.

**Effort prorated among corn, wheat, and grain sorghum.

and to the University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968). During the year, research on glucopyranose rings in starches and dextrins was completed at the "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy.

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); to Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); to Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); to Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); to University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); to the Institute for Fibres and Forest Products, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968); and to the University of Graz, Graz, Austria, for rheological studies on aqueous dispersions of modified cereal starches and paper coating formulations containing starch-based adhesives (3 years, 1966-1969). During the year, research was completed on phosphorus- and sulfur-containing cationic starches at the National Institute of Technology, Rio de Janeiro, Brazil.

Research on microbiology and fermentation involves grants to the University of Allahabad, Allahabad, India, for studies on survival of lyophilized microorganisms (5 years, 1962-1967); to Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India to find new accessions for the ARS Culture Collection (5 years, 1965-1970); to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); to the University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and of mevalonic acid (3 years, 1965-1968); to the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and to the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969). During the year, research was completed on collection of new Mucorales species at the University of Allahabad, Allahabad, India; on investigations of sugar phosphate derivatives in molds at the University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England; and on studies on the preparation and characterization of dextran derivatives at the University of Rome, Rome, Italy.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of .6 scientist man-year is devoted to research on industrial and feed uses of wheat.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Characterization of wheat gluten proteins. Studies on the structure of wheat proteins resulted in isolation of one pure α-gliadin, three other α-gliadins and two β-gliadins in nearly pure condition by separation of whole gliadin on sulfoethyl cellulose and Sephadex. Larger quantities of γ_1, γ_2, and γ_3-gliadins were also prepared. A comparative chromatographic and electrophoretic study of gliadin proteins from 10 wheat varieties representing four classes of wheat showed that proteins from varieties within the same class were similar, although not identical. Differences between classes were substantially greater. Two HRW wheats that have different baking properties showed only small differences in gliadin protein content. Each variety analyzed contained a gliadin component comparable to the γ_1-gliadin of Ponca wheat. These γ_1-gliadins have nearly identical amino acid compositions. Studies on gel-permeation chromatography of glutenin, cyanoethyl glutenin and gliadin in 8 M urea on polyacrylamide gel showed that reduced and alkylated glutenin proteins behave as particles that have apparent molecular weights near 100,000 and 40,000 in strongly deaggregating solvent.

In studies on physical chemistry of wheat gluten, it was found that when gliadin was freed of its high-molecular-weight component, the remainder showed no drop in viscosity accompanying cleavage of disulfide bonds. The conclusion is that the high-molecular-weight component contains intermolecular disulfide bonds, whereas the low-molecular-weight material contains only intramolecular disulfide bonds. Evidence was obtained that the slow secondary increase in viscosity observed after cleavage of disulfide bonds of gluten and gliadin in ethanol-acetic acid solution is caused by aggregation of unfolded peptide chains. No such increase was observed in the presence of 8 M urea. The optical rotatory dispersions of glutenin and gliadin in trifluoroethanol (helix-forming solvent) and in hexafluoroacetone sesquihydrate (random-coil forming solvent) were studied. Glutenin has 36 percent helix and gliadin has 43 percent helix in trifluoroethanol. When glutenin in trifluoroethanol was dialyzed against 0.01 N HAc, the amount of helix became 14 percent, which is the same as for glutenin in 0.01 N HAc. Helix content of glutenin and gliadin in the sesquihydrate is reduced to 11-12 percent. Optical rotatory dispersion results indicate the possibility of substantially changing the conformations of glutenin and gliadin by the proper choice of solvents.

2. Chemistry of glycoprotein linkages. Under a grant to Marquette University, glycoprotein investigations have now resulted in the synthesis of an

O-glucosaminide of 4-hydroxyproline. Unlike the earlier synthesized serine and threonine analogs, the new glucosaminide was stable to alkali. Five new O-glycosides of hydroxyamino acids have now been synthesized and characterized. Determination of the stability of these compounds toward hydrolytic conditions and solvents has given information on the stability of glycosidic linkages in glycoproteins.

3. Microscopic and ultrastructure of wheat grain. Research on microscopic and ultrastructure of wheat grain and on changes induced therein by various treatments is relevant to industrial utilization of wheat. Results are reported under "Wheat Utilization - Food," subheading A-1.

4. NMR studies. Nuclear magnetic resonance techniques are employed in studies relevant to industrial utilization of wheat. Results are reported in "Corn Utilization - Industrial Products," subheading A-3.

B. Chemical and Physical Investigations to Improve Products

1. Chemical modification of wheat gluten. Studies on chemical modification of reduced wheat proteins showed that sulfhydryl groups could be selectively modified by reaction with acrylonitrile, acrylamide or methyl acrylate. A new program for computer processing of amino acid analysis data has been successfully written and is being utilized. Sodium hydride in DMSO was shown to be a good reducing system for protein disulfide bonds. Results to date indicate that reduction proceeds without peptide cleavage or desulfurization. Decrease in lysine residues in bovine serum albumin and whole gluten proteins treated with an excess of acrylonitrile appears to follow pseudo-first-order kinetics.

2. Polypeptide derivatives. In studies under a contract to IIT Research Institute on the chemical modification of cereal polypeptides, it was found that films and fibers from epoxidized and ethyleneimine-treated gluten hydrolyzates had low tensile strengths. However, these materials showed excellent adherence to metal surfaces. A transparent water-soluble film having good tensile strength was obtained from ethyleneimine-treated hydrolyzate to which neutralized hydrolyzate had been added. Derivatives of whole gluten or of gluten hydrolyzates have been prepared and evaluated as adhesives and plastics. Polymerization of ethylene oxide in gluten hydrolyzate was shown to proceed via reaction with free amino groups. Grafting of polyoxyethylene chains to wheat gluten hydrolysis products gave materials having lower solution viscosities and sedimentation coefficients than did the untreated polypeptides. When the modified and original polypeptide fractions were subjected to gel filtration on Sephadex G-25, the original polypeptide was completely excluded from gel matrix. The modified protein contained a large fraction that was retarded. It was concluded that some degradation of polypeptide occurs and some polyethylene oxide homopolymer forms during the modification reaction. Tests of the polyethylene oxide-modified gluten hydrolyzate as a plasticizing agent for gluten indicated that films made from equal amounts of gluten and the copolymer were quite flexible.

3. Studies on wheat starch. Chemical and physical investigations on wheat starch are integrated with similar research on corn starch reported under "Corn Utilization - Industrial Products," subheading B.

C. Microbiology and Fermentation

Research on microbiological and fermentative processes for converting wheat starch or flour to industrial products is integrated with similar studies on corn. Results are reported under "Corn Utilization - Industrial Products," subheading C.

D. Technology--Process and Product Development

1. Acid-modified flour (AMF). Contract research involving experimental work on acid-modified flour (AMF) as a surface-sizing agent for paper has been completed by the Brown Company. Results show that strength and surface properties of paper sized with AMF and with commercial reference starch were similar. Protein content of the size paste remaining after 80 percent of the paste had been consumed was 14 percent, as compared to an initial value of 8 percent. Operating conditions and product properties were not adversely affected by this degree of protein buildup.

2. Other developmental research. Development of products and processes involving wheat starch is integrated with related work on corn starch. Results are given under "Corn Utilization - Industrial Utilization," subheading D.

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AREA NO. 5: WHEAT UTILIZATION - FOOD (NORTHERN REGION)

Problem. The dominant factor in the wheat economy of the United States continues to be a production capacity that can outpace consumption, including the substantially expanded foreign markets of recent years. Increased exports of wheat from the United States in the last 3 years have brought our wheat carryover to a level that provides less than a prudent reserve. However, the capacity to produce wheat in this country is still restricted.

We view this North American surplus capacity as an unparalleled opportunity. Wheat in excess of domestic needs can be used to buy time in the overpopulated areas of the world until a self-sufficient agriculture can be developed there. Export donations and concessional sales of 600-800 million bushels per year are providing food where it is most needed in the world. This distribution of wheat serves immediate Defense and State Department missions, and also stimulates a long-range market development for United States agriculture. New wheat foods specifically adapted to conditions of use in every region of the world would help materially to popularize this valuable food grain in areas where it is now virtually unknown, and development of simplified methods to process the products at the point of use would speed their adoption.

We also need to increase the commercial exports (currently less than 200 million bushels annually) that contribute favorably to our international trade balance. New processes to elicit maximum quality performance of wheats and flours in products produced in Europe and Japan would help significantly to promote trade in these dollar markets. Sustained further gains in wheat markets are necessary to ease governmental restrictions on production more than they have already been eased, and especially to strengthen export trade balances. Increased world supplies of wheat and restrictive political decisions in the European Economic Community have contributed to seriously reduced commercial exports in some years. Everything possible must be done to increase total wheat markets, but especially those in which payments are made in dollars.

Consumers of wheat foods in this country have benefited greatly by introduction of a wide variety of new and improved products. Well-balanced diets, reasonable food costs, and improved convenience result from such developments and are suitable objectives of research. Domestic per capita consumption has become stabilized over the past 3 years. Research programs along these lines thus sustain and increase markets for wheat.

An essential foundation for a successful product and process development program is basic research on the composition of all classes of wheat and the fundamental properties of their constituents. This kind of information provides the foundation for improved and new products and processes.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of wheat in food.

The Federal scientific effort (Northern region) for research on food utilization of wheat totals 7.9 scientist man-years. Of this number, 3.1 are devoted to chemical composition and physical properties; 4.2 to microbiology and toxicology; and .6 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (2.1 scientist man-years) includes studies on separation and physical and chemical characterization of wheat proteins and on the microscopic and ultrastructure of wheat grains and flours and the effects of various treatments. A research contract (1.0 scientist man-year) is in effect with the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on varietal variations in kernel properties and milling and fractionation characteristics of wheat.

Research at Peoria, Illinois, on microbiology and toxicology (4.0 scientist man-years) is concerned with development of new fermented foods from wheat, with reduction of the microbial population of wheat and wheat flour and with studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.2 scientist man-year) is allocated to research on food uses of wheat.

Research conducted at Peoria, Illinois, on technology--process and product development (.6 scientist man-year) comprises investigations on modified techniques for milling and fractionation of wheat to obtain improved products for food. During the year, milling research was redirected to emphasize discovery and evaluation of new and unconventional approaches. Investigations on reducing radioactive contamination of wheat and wheat flour were successfully completed.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 14.8 scientist man-years is devoted to research on food uses of wheat.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Microscopic and ultrastructure of wheat. Studies have been initiated on the role of lipids in formation of gluten strands. Results to date indicate that native wheat protein produces much longer strands than does protein from which lipids have been extracted. Direct electron microscopy of wheat protein strands revealed that, in most cases, strands formed with flour particles on water surfaces were not sufficiently thin to provide adequate resolution. Numerous spherical electron dense bodies are frequently observed in association with fixed protein strands; the nature of these bodies and the conditions which lead to their appearance are not as yet clear. The strands were markedly reduced in length in all cases where flour particles had been previously defatted. Extensibility of wheat protein on tap water was appreciably greater than on distilled water. SRW wheat (Vermillion) forms finely subdivided protein strands in contrast to relatively coarse strands formed by HRW (Wichita) or HRS (Thatcher) wheats.

2. Crosses of soft and hard wheats. Contract studies on milling crosses between hard and soft Nebraska wheats at the University of Nebraska showed that treatment of hard wheat with enzymes specific for pectin and proteolytic enzymes resulted in milling properties resembling those of soft wheat. Previous conclusion that acid-soluble pentosans are not involved in milling efficiency still stands. Kernel penetration hardness tests or visual estimates of kernel vitreousness have little correlation with actual milling properties, whereas microscopic observation of endosperm breakdown caused by crushing a small specimen between two slides appears more reliable. Differences in relative amounts of protein fractions recovered by the Maes procedure indicate that mixing of dough prior to extraction introduces structural changes in flour proteins. Milling type could not be correlated with the results of the Maes procedure as modified to give improved electrophoretic patterns.

3. Characterization of wheat gluten proteins. Results, reported under "Wheat Utilization - Industrial Products," subheading A-1, are important to understanding the unique functional properties of wheat flour.

B. Microbiology and Toxicology

1. Reduction of viable microorganisms in flour and flour products. Dominant bacterial flora of domestic wheats and flours is, like that of the molds, limited to only a few genera. Total bacteria counts of wheats and flours from Texas-Oklahoma and southeastern U. S. were, in general, lower than those of samples from northern wheat-producing areas. Lactic acid bacteria constituted a very small part of the microflora of domestic wheat and flour. A practical method has been developed for producing flour with little protein damage and low microbial count from highly contaminated

wheat. Method involves steaming wheat for 15 seconds, quenching in cold water containing 250 p.p.m. of chlorine, dewatering, drying, and milling. The procedure can be used on a continuous basis and can be readily integrated into most mill flows. Studies on the microorganisms in dry-milled corn products have revealed a situation essentially analogous to that observed for wheat flour and milled fractions. Three potential processes for reducing microbial count in dry-milled corn products are being evaluated.

2. Fermented wheat foods. Rat-feeding experiments indicated that tempeh fermentation did not increase the protein efficiency ratio of soybean protein but did greatly increase the PER of wheat. No evidence of any kind of toxicity was found in these experiments. Diets containing a mixture of wheat and soybeans, either fermented or unfermented, as a protein source resulted in good body weight gain comparable to that of the reference casein diet. PER of the fermented mixture was essentially equivalent to that of casein. The patterns of essential amino acids released from wheat and fermented wheat by trypsin-pancreatin digestion reveal that greater amounts of lysine and histidine are released from fermented wheat than from control wheat. Satisfactory tempeh has been made from cracked low-fat peanuts and pressed peanuts. The majority of the tempeh mold strains were also found to produce a rennin-like enzyme which probably is the same proteolytic enzyme system previously reported.

3. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of wheat in food. Results are reported under "Corn Utilization - Feed," subheading B-1.

C. Technology--Process and Product Development

1. Milling and fractionation. Vacuum conditioning and concentrated spray treatment are both suitable means for lysine fortification of whole-grain wheat which is to be milled to 95 percent extraction for human consumption. Adding 2 percent lysine to wheat, the 95 percent extraction flour retained 82 percent of the added lysine. After applying 0.2 percent lysine by direct spraying, 69 percent of the added lysine was retained by the flour. Defatted, first oat flour (9.8% protein) showed a 50 percent protein shift, slightly more than for second oat flour (20% protein). These flours resemble hard wheat flour in response to air classification. Yield of high-protein first fraction was unusually large. The use of high temper moisture (22%) and prebreak milling, to help loosen protein from starch of HRW wheat, produced a small increase in break flour and overall flour yields.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Microbiology and Toxicology

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Technology--Process and Product Development

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*Research supported by PL 480 funds.

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Kent, N. L. (Research Association of British Flour-Millers, St. Albans, Herts., England). 1966. Subaleurone endosperm cells of high protein content. Cereal Chem. 43(5), pp. 585-601.*

Kent, N. L., and Evers, A. D. (Research Association of British Flour-Millers, St. Albans, Herts., England). 1966. Endosperm reduction in hard red spring wheat. Northwest. Miller 273(12), pp. 12, 14, 16, 18, 20-22.*

Stringfellow, A. C., Peplinski, A. J., and Pfeifer, V. F. 1967. Fine grinding of wheat millfeeds and whole wheat for industrial use. Cereal Sci. Today 12(2), pp. 43-45, 48, 60.

*Research supported by PL 480 funds.

AREA NO. 6: WHEAT UTILIZATION - FEED (NORTHERN REGION)

Problem. In the last 2 years the use of wheat for feed increased to nearly 100 million bushels per year, more than twice the amount used in any other recent year. Unfortunately, wheat has certain performance drawbacks as a feed. Research that develops new processes to improve feeding quality of wheat will benefit both growers and feeders in wheat-producing areas, since it will place this grain in a more competitive position as compared with other grains, and it can reduce freight costs.

Millfeeds are not used extensively in modern poultry and swine rations because the high fiber content cannot be tolerated in high-energy rations. If inexpensive ways of separating low-fiber, high-protein fractions from millfeeds are developed, these new materials can be used as protein and energy sources for nonruminant diets, and the overall value of milling by-products will be increased. Flour production is expected to increase in the near future to reflect the demands of our increasing domestic population and of the new export markets which are developing. More milling will result, of course, in more millfeeds. If these millfeeds cannot be utilized efficiently and effectively, the price for flour will have to increase to carry the economic burden.

Meat production, particularly poultry, is increasing rapidly in Japan and the European Economic Community where modern efficient methods have been introduced. This development depresses the opportunity for exporting poultry and other meats into these important trade areas, but it offers an increasing opportunity to sell feeds. Upgrading of wheat millfeeds through utilization research will increase our export markets.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of wheat in feed.

The Federal scientific effort (Northern region) for research on utilization of wheat in feeds totals 1.8 scientist man-years, of which .3 is devoted to chemical composition and physical properties; 1.2 to microbiology and toxicology; and .3 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (.3 scientist man-year) involves basic investigations of the microscopic and ultrastructure of wheat grains and the effects of various treatments.

Research at Peoria, Illinois, on microbiology and toxicology (1.0 scientist man-year) is concerned with studies on the production of mycotoxins by Aspergillus flavus and related molds. A research contract (.2 scientist man-year*) is in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites. During the reporting period, research was completed at A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene, and with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene.

Research conducted at Peoria, Illinois, on technology--process and product development (.3 scientist man-year) is concerned with studies on modified techniques of milling and fractionation to obtain improved products. During the year, milling research was redirected to emphasize discoveries and evaluations of new and unconventional approaches. Investigations on reducing radioactive contaminants of wheat and milled fractions were successfully completed.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains (4 years, 1966-1970). Effort on this project is prorated among corn, wheat, and sorghum.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of .6 scientist man-year is devoted to research on industrial and feed uses of wheat.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Studies on the microscopic and ultrastructure of wheat are relevant to utilization of wheat in feeds. Results are reported under "Wheat Utilization - Food," subheading A-1.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of wheat in feeds. Results are reported under "Corn Utilization - Feed," subheading B-1.

*Work covers more than one commodity; only effort allocated to wheat is included in total.

C. Technology--Process and Product Development

Research on milling and fractionation of wheat and on reduction of radioactive contamination in wheat and milled products is relevant to utilization of wheat in feeds. Results are reported under "Wheat Utilization - Food," subheading C.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 7: GRAIN SORGHUM UTILIZATION INDUSTRIAL PRODUCTS

Problem. The growing importance of grain sorghum as a competitive crop is revealed by the record 655-million-bushel crop in 1965. About 80 percent of the grain sorghum crop is grown in Texas, Kansas, and Nebraska. Sorghum starch and flour find industrial usage where freight transportation advantages exist. Currently an estimated 6 million bushels of sorghum are milled for products consumed mainly by the paper and gypsum board industries. To maintain this market against competition from synthetics and to take advantage of opportunities that exist in economically favorable geographic areas for increased industrial utilization of sorghum, technology suited to the specific characteristics of this grain and its milled products must be developed.

Research on sorghum starch is integrated with that on corn starch. A more detailed discussion of promising industrial outlets and of the pertinent research required is given under Area 1, Corn Utilization - Industrial Products. Because grain sorghum has a round kernel in contrast to the odd-shaped kernel of corn, it lends itself to dry milling innovations not possible with corn. Milling improvements, together with the possible advantages of air classification of sorghum flour, offer prospects for increasing industrial utilization by making processing economics more attractive and by providing products with superior properties.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of grain sorghum, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of grain sorghum totals 9.6 scientist man-years. Of this number, .5 is devoted to chemical composition, physical properties and structure; 3.1 to chemical and physical investigations to improve products; 4.1 to microbiology and fermentation; and 1.9 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.3 scientist man-year) involves study of applications of nuclear magnetic resonance spectroscopy to grain components and is integrated with related research on corn. During the year, studies on the rheological properties of starch were initiated. Contract research was completed by Indiana University Foundation, Bloomington, Indiana, for studies on the isolation and characterization of phenolic pigments of

grain sorghum. Grants (.2 scientist man-year*) are in effect with Iowa State University, Ames, Iowa, for basic research on heat, mass, and momentum transport of cereal starches and flours; and with the State University of New York, Syracuse, New York, for investigations of starch fine structure.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (1.9 scientist man-years) is integrated with research on corn starch and is directed to wide-ranging study of the chemical reactions of starch with the objective of discovering new chemical products and processes having potential for industrial use. During the year, studies on starch-based plastifoams were completed and research on related noncellular plastics was initiated. Research contracts (.5 scientist man-year*) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of acetylene with methyl glucoside; with the University of Akron, Akron, Ohio, for evaluation of starch and starch derivatives as reinforcing agents for natural and synthetic rubber; with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides; with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper; and with General Mills, Central Research Laboratories, Minneapolis, Minnesota, for studies on the development of cereal proteins having utility as flotation and flocculating agents. Contract research was completed by The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media, and with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers. Grants (.7 scientist man-year*) are in effect with Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; with Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; with Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus, and sulfur; with the University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; with the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides; and with Southern Illinois University, Carbondale, Illinois, for studies on the alcoholysis of carbohydrate esters.

Research on microbiology and fermentation conducted at Peoria, Illinois, (3.3 scientist man-years) includes studies on the use of microorganisms to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. This research is integrated with similar studies based on corn. A large collection of pure cultures of industrially and agriculturally important microorganisms is

*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

maintained. The Pioneering Laboratory for Microbiological Chemistry conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (.2 scientist man-year*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation; at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective; and at Michigan State University, East Lansing, Michigan, for investigation of the biochemical properties of variant cultures of Bacillus popilliae. Contract research at the Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms has been completed. Grants (.6 scientist man-year*) are in effect at Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; at Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; at Iowa State University, Ames, Iowa, for investigation of bacterial amylases and their action patterns; at the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; at the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch; at the University of Nebraska, Lincoln, Nebraska, for structural studies of fungal glucohydrolases; at Baylor University, Houston, Texas, for cytology of ascospore formation in yeasts; at the University of Minnesota, Minneapolis, Minnesota, for studies on cellular differentiation and physiology of selected molds; at East Texas State University, Commerce, Texas, for determinations of branching in polysaccharides; and at Indiana State University, Terre Haute, Indiana, for surveys of gum-producing microorganisms. During the year, grant research was completed by Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes.

Research conducted at Peoria, Illinois, on technology--process and product development (1.4 scientist man-years) is concerned with detailed study and evaluation of starch derivatives having definite potential for industrial utilization and of processes for making them. The work is integrated with similar studies on corn starch derivatives. Research contracts (.5 scientist man-year*) are in effect at Stanford Research Institute, Menlo Park, California, for process development of selected starch graft copolymers; at Western Michigan University, Kalamazoo, Michigan, for evaluation of

*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

modified cyanoethylated starches for applications in paper; at Battelle Memorial Institute, Columbus, Ohio, for studies on starch derivatives for use as colloids in water-emulsion paints; and at Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations on the use of starch glycosides in coatings and plastics. During the year, contract research on starch and other cereal grain xanthides was completed by Battelle Memorial Institute, Columbus, Ohio.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds.* Research on chemical composition, physical properties and structure involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); and to the University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968). During the year, research on glucopyranose rings in starches and dextrins was completed at the "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy.

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); to Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); to Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); to Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); to the University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); to the Institute for Fibres and Forest Products Research, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968); and to the University of Graz, Graz, Austria, for rheological studies on aqueous dispersions of modified cereal starches and paper coating formulations containing starch-based adhesives (3 years, 1966-1969). During the year, research was completed on phosphorus- and sulfur-containing cationic starches at the National Institute of Technology, Rio de Janeiro, Brazil.

Research on microbiology and fermentation involves grants to the University of Allahabad, Allahabad, India, for studies on survival of lyophilized microorganisms (5 years, 1962-1967); to Central Drug Research Institute,

*Effort prorated among corn, wheat, and grain sorghum.

Lucknow, India, for studies on aerobic actinomycetes in India to find new accessions for the ARS Culture Collection (5 years, 1965-1970); to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); to the University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and of mevalonic acid (3 years, 1965-1968); to the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and to the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969). During the year, research was completed on collection of new Mucorales species at the University of Allahabad, Allahabad, India; on investigations of sugar phosphate derivatives in molds at the University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England; and on studies on the preparation and characterization of dextran derivatives at the University of Rome, Rome, Italy.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of .3 scientist man-year is devoted to research on industrial and feed uses of grain sorghum.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. NMR studies. Nuclear magnetic resonance techniques are employed in studies relevant to industrial utilization of grain sorghum. Results are reported under "Corn Utilization - Industrial Products," subheading A-3.

B. Chemical and Physical Investigations to Improve Products
C. Microbiology and Fermentation
D. Technology--Process and Product Development

Research in these categories is integrated with similar investigations on corn starch. Results are reported under "Corn Utilization - Industrial Products," subheadings B, C, and D.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Technology--Process and Product Development

Stringfellow, A. C., and Peplinski, A. J. 1966. Air classification of sorghum flours from varieties representing different hardnesses. Cereal Sci. Today 11(10), pp. 438-440, 445.

AREA NO. 8: GRAIN SORGHUM UTILIZATION - FOOD

Problem. An estimated 4 million bushels of grain sorghum are utilized annually in products for human consumption. This sorghum includes some special varieties such as white and waxy sorghums. Sorghum starch and derived glucose and glucose sirup are used in foods, and sorghum grits are used in fermented beverages. Although this outlet is at present quite limited, the growing importance of grain sorghum as a cash crop in the Southwest indicates that opportunities for increasing food use of sorghum should not be overlooked. Since grain sorghum is a staple food in many parts of Asia and Africa, a further consideration is the development of food products that could contribute to alleviation of dietary deficiencies in many developing countries.

To achieve the objective, more information on the composition of grain sorghum is needed. For example, some varieties contain pigments that can discolor milled products and that may contribute to undesired flavors. Questions have been raised concerning the digestibility and nutritive value of sorghum protein that reveal the need for better data on amino acid composition and on minor constituents.

Milling innovations, such as tangential abrasion, make possible conversion of about 20 percent of the sorghum kernel to a flour containing 25 percent protein. This and other possible approaches to new food products should be evaluated.

It has recently been discovered that certain oilseeds and cereal grains, including sorghum, are subject to infection by molds that can produce toxic products. To provide safe food products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from sorghum.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of grain sorghum in food.

The Federal scientific effort for research on food utilization of grain sorghum totals 6.4 scientist man-years. Of this number, 2.6 are devoted to chemical composition and physical properties, .3 to microbiology and toxicology, and 3.5 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (2.4 scientist man-years) involves studies on properties of grain sorghum proteins. Contract research is in effect with Kansas State University, Manhattan, Kansas, for investigations on the composition, processing, and feeding value of hybrid grain sorghum. A portion of this effort (.2 scientist man-year) is allocated to research on food uses of grain sorghum.

Research at Peoria, Illinois, on microbiology and toxicology (.2 scientist man-year) is devoted to studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.1 scientist man-year) is allocated to research on food uses of grain sorghum.

Research conducted at Peoria, Illinois, on technology--process and product development (3.5 scientist man-years) is devoted to the development of new and improved processing techniques to obtain nutritious food products.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.0 scientist man-years is devoted to research on food uses of grain sorghum.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nutritional quality of grain sorghum. Contract studies on composition, processing, and feeding value of hybrid grain sorghums are important to food utilization of sorghum. Results are reported under "Grain Sorghum Utilization - Feed," subheading A-1.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of grain sorghum in foods. Results are reported under "Corn Utilization - Feed," subheading B-1.

Preliminary studies on the performance of a variety of machines as they are applied to the dry dehulling and degermination of grain sorghum resulted in recoveries of +20 grits of 68-75 percent, with fat contents of these grits ranging from 0.4 to 0.8 percent. Best overall results were obtained from the solid rotor machine developed in the Engineering Laboratory at NU. However, all treatments--which included impaction, pearling plus impaction, dehulling in a rice huller plus impaction, and the use of a brush device--produced grits which were somewhat comparable to commercial sorghum brewers'

grits. A range of conditions was established for the partial gelatinization on heated rolls of grain sorghum flour and grits to produce products with different viscosity and other properties. This study revealed that the moisture content of the flour or grits was the most critical factor in effecting different degrees of gelatinization, with the temperature next. At higher moisture levels the temperature effect was more pronounced, while the roll speed played a lesser role. Particle size of the flour or grits did not appear to have a great effect under any conditions of gelatinization tested. Alkali dehulling was applied successfully to grain sorghum, although a discoloration remained at the tip cap of each kernel after treatment. Alkali requirements are considerably reduced for sorghum as compared to wheat.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 9: GRAIN SORGHUM UTILIZATION - FEED

Problem. The principal domestic use of grain sorghum produced in the U. S. is as feed for animals. The record 655-million-bushel crop in 1965 reveals the growing importance of this grain. About 80 percent of the crop is grown in Texas, Kansas, and Nebraska.

Problems are encountered in the use of grain sorghum in feeds which, if solved, could increase utilization and economic value of this crop to farmers and to the feed industry. The major need is for more and better information on the protein content and amino acid composition of various varieties of grain sorghum as related to biological feeding value. Minor constituents having physiological activity also require more adequate study. For example, certain phenolic pigments may impart bitterness and thereby reduce palatability. Carotenoid pigments, which in part are precursors for Vitamin A, are valuable in poultry rations for imparting yellow color to egg yolks and to the skin of fryers and broilers. In addition to such compositional studies, processing investigations are needed to provide ways for preserving desired and removing undesired components. Sorghum is included in the group of cereal grains and oilseeds recently recognized to be subject to infection by molds capable of producing toxic products. To provide safe feed products and to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from grain sorghum.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of grain sorghum in feed.

The Federal scientific effort for research on utilization of grain sorghum in feed totals 1.0 scientist man-year, of which .7 is devoted to chemical composition and physical properties and .3 to microbiology and toxicology.

Research on chemical composition and physical properties involves a research contract in effect with Kansas State University, Manhattan, Kansas, for investigations on the composition, processing, and feeding value of hybrid grain sorghums. A portion of this effort (.7 scientist man-year) is allocated to research on feed uses of grain sorghum.

Research at Peoria, Illinois, on microbiology and toxicology (.2 scientist man-year) is concerned with studies on the production of mycotoxins by

Aspergillus flavus and related molds. A research contract (.1 scientist man-year*) is in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites. During the reporting period, research was completed at A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene, and with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on chemical composition and physical properties involves a grant to the Indian Institute of Science, Bangalore, India, for research on separation of grain sorghum proteins (5 years, 1963-1968).

Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains (4 years, 1966-1970). Effort on this project is prorated among corn, wheat, and sorghum.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of .3 scientist man-year is devoted to research on industrial and feed uses of grain sorghum.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nutritional quality of grain sorghum. In studies on the nutritional quality of sorghum proteins, conducted under a contract with Kansas State University, eight diets, each based on a different sample of 1965 crop sorghum, were fed to chicks over a period of 4 weeks. Each diet contained 15 percent of protein and consisted of 78.5 percent sorghum, 10 percent soybean meal, and small amounts of other needed ingredients. Compositional differences among the sorghum samples were disappointingly low because of an unusual growing season. Weight gains for the chicks were not significantly altered by different sorghum hybrids; by location where sorghum was grown; or by nitrogen fertilization or irrigation of growing sorghum. Addition of pure lysine to these diets significantly increased weight gain, but no significant change occurred with methionine. Therefore, methionine may not be the first limiting amino acid as thought previously. Feeding experiments are currently underway using sorghum from the 1966 crop. This crop is similar to the 1965 crop in that variety, location, fertilizer level, and irrigation appear to have had minimum effect on protein content.

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*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

2. Studies on sorghum proteins. Under a PL 480 grant at the Indian Institute of Science, lysine determinations have been completed on 44 varieties of sorghum of world-wide origin and five Indian varieties. With only one exception, lysine content of protein showed a negative correlation with protein content. Complete amino acid analyses by ion exchange chromatographic procedures for six genetic varieties and three hybrid varieties of sorghum seed representing high and low protein levels indicate that prolamine and glutelin are the major protein fractions and that variations in the total amount of protein are primarily attributable to differences in the prolamine content. Amino acid composition studies of the various protein fractions are continuing.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of grain sorghums in feeds. Results are reported under "Corn Utilization - Feed," subheading B-1.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 10: SOYBEAN UTILIZATION INDUSTRIAL PRODUCTS

Problem. As an industrial oil, soybean oil is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. Largely because of effective research, nonfood usage of soybean oil has rather consistently accounted for about 10 percent of domestic disappearance. The best opportunity for maintaining or increasing industrial applications of soybean oil is to be found in development of nontraditional products that can compete with synthetics in the multibillion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the objective, more fundamental information is needed on reactions of soybean oil, especially those that will preserve the glyceride structure, and on the physical and chemical properties of the products.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing, long-range program involving analytical, organic, and physical chemists, and chemical engineers engaged in basic and applied research to obtain new information on chemical reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries. In addition, microbiologists are engaged in a limited study of the possibilities of fermentative modification of fatty acids derived from soybean oil.

The Federal scientific effort for research on industrial utilization of soybean oil totals 11.1 scientist man-years. Of this number, .8 is devoted to chemical composition, physical properties and structure; 7.6 to chemical and physical investigations to improve products; 1.7 to microbiology and fermentation; and 1.0 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.8 scientist man-year) is devoted to mass spectrometric investigations of chemical and molecular structure of glyceride oils and their derivatives.

Research on chemical and physical investigations to improve products in progress at Peoria, Illinois (7.2 scientist man-years), emphasizes studies of aldehyde derivatives of soybean oil. A research contract with the University of Illinois, Urbana, Illinois, provides for basic studies on the mechanism of homogeneous hydrogenation with organometallic catalysts. A portion of this effort is allocated to industrial utilization of soybean oil (.4 scientist man-year). A research contract with North Dakota State

University of Agriculture and Applied Science, Fargo, North Dakota, for investigations of aldehyde oils as components of protective coatings was completed.

Research at Peoria, Illinois, on microbiology and fermentation (1.7 scientist man-years) involves exploration of possibilities for producing industrially useful derivatives by microbial conversion of fatty acids.

Research on technology--process and product development involves a research contract (1.0 scientist man-year*) in effect with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils. During the reporting period, the Archer Daniels Midland Company, Minneapolis, Minnesota, completed contract research involving pilot preparation of various aldehyde oil products needed for developmental investigations.

The Department also sponsors research in this area under grants of PL 480 funds to foreign institutions. Under the heading chemical and physical investigations to improve products, research** was completed under a grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on stereospecific polymerization of polyunsaturated fatty esters.

Research on microbiology and toxicology involves a grant to the University of Baroda, Baroda, India, for studies** on production of microbial lipases useful for modifying vegetable oils (5 years, 1965-1970).

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Mass spectroscopy. Mass spectrometric investigations of chemical and molecular structure of glyceride oils and their derivatives are relevant to industrial utilization of soybean oil. Results are reported under "Soybean Utilization - Food," subheading A-1.

B. Chemical and Physical Investigations to Improve Products

1. Aldehyde oil derivatives. A survey of possible alternative procedures for ozonolysis and decomposition of ozonolysis products revealed none giving

*Work covers more than one commodity; only effort allocated to soybeans is included in total.

**Effort prorated between linseed and soybeans.

higher carbonyl yields than the presently used procedure (ozonolysis in a reactive solvent followed by chemical or catalytic reduction). Ethyl and butyl azelaaldehydate were prepared in high purity by simplified procedures. These esters are more resistant than the methyl derivative to hydrolysis, ammonolysis and aminolysis. High yields of methyl 9-hydroxynonanoate (potential intermediate for polyesters) were obtained by catalytic hydrogenation of ozonolysis products of soybean methyl esters. 9-Aminononanoic acid (nylon-9 intermediate) was prepared in overall yields of more than 60 percent from soybean butyl esters via the azelaaldehydate route. Yields of high-purity amino acid as high as 94 percent were obtained by reductive alkylation of ammonia with methyl (MAZ) and ethyl azelaaldehydates followed by hydrolysis of the unisolated amino ester. The amino acid was polymerized by heating without catalyst to give a nylon-9 polymer. Poly(ester-acetals) and poly(amide-acetals) were successfully used as polar "liquid" phases for GLC columns thermally stable to 350° C. A group of poly(ester-acetals) prepared by bulk polymerization of MAZ glyceryl acetal (MAZGA) with a variety of catalysts have been characterized to elucidate their properties and potential as intermediates. A tough, elastic crosslinked polymer was obtained by heating an MAZGA poly(ester-acetal) with p-toluenesulfonic acid and zinc oxide.

2. Cyclic fatty acids. Studies on the preparation of cyclic fatty acids are relevant to industrial utilization of soybean oil. Results are reported under "Flax Utilization - Industrial Products," subheading B-1.

C. Microbiology and Fermentation

1. Microbial modification of fatty acids. The pseudomonad NRRL P-1151, now NRRL B-3266, oxidizes oleic acid to a mixture of 10-ketostearic and 10-hydroxystearic acids. Addition of minimal amounts of oleic acid during growth of the bacterium provides more rapid conversion of oleic acid to product. No enzyme activity was detected in the growth medium. Complete utilization of the substrate oleic acid appears to shift the ratio of products toward the keto acid, suggesting that the hydroxystearic acid is an intermediate. Fermentation under anaerobic conditions showed that 10-hydroxystearic acid is produced preferentially when oxygen is absent, whereas 10-ketostearic acid is major product when oxygen is present. Continued incubation after complete utilization of oleic acid results in degradation of the initial oxidation products by chain shortening. Two products arising from beta oxidation of 10-ketostearic and 10-ketopalmitic acid by B-3266 have been identified as 4-ketolauric and 6-ketolauric acid, respectively. Degradation of the 10-hydroxy fatty acids has been eliminated by use of anaerobic fermentation conditions. Oxygen-containing products are formed by fermentation of linoleic or linolenic acids with B-3266 under anaerobic conditions. Presumably, the products are the corresponding 10-hydroxy unsaturated acids.

Under a PL 480 grant at the University of Baroda, Baroda, India, several strongly lipolytic bacteria have been isolated from sewage. A strain of

Pseudomonas aeruginosa has been extensively investigated. This organism produces a lipase when grown on a medium of salts, vitamins, glycerol, casein hydrolyzate and yeast extract. The level of glycerol is critical and 0.2 percent is optimum. Likewise, 0.2 percent casein hydrolyzate is optimum. The best medium for lipase production differs from a medium for maximum growth, since growth is greater when larger amounts of glycerol and casein are used. The lipase was isolated from the organism in the form of acetone powders. The enzyme showed optimum activity at pH 7.0 when run at 25 to 28° C. Calcium ion was found to activate the enzyme. Ninety percent of a soybean oil substrate was hydrolyzed in 3 hours with the production of free fatty acids plus lesser amounts of mono and diglycerides.

D. Technology--Process and Product Development

1. Aldehyde oils and derivatives. In the fundamental investigation of the crosslinking reaction involving poly(ester-acetals) and poly(amide-acetals), evidence was obtained that the size of the acetal ring influenced the reactivity of the poly(ester-acetal) during the reaction. However, the effect of ring size was not as great as hoped. The use of a promoter such as 3,3-bis-(chloromethyl)oxetane in combination with certain metal catalysts permitted crosslinking to occur at relatively mild conditions of 150° C. and 1 to 3 hours. Copolymers with 50 percent of diglycerol acetal of methyl azelaaldehydate and nylon-6 pentaerythritol acetal copolymers are being prepared for testing as industrial products. These studies are being conducted under a contract with Fabric Research Laboratories.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Investigations to Improve Products

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*Research supported by PL 480 funds.

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Technology--Process and Product Development

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*Research supported by PL 480 funds.

AREA NO. 11: SOYBEAN UTILIZATION - FOOD

Problem. Worldwide shortages of dietary protein and of food fats pose a problem that urgently demands solution. Since soybeans can furnish both of these nutritionally essential substances, foreign markets provide a promising outlet for the rapidly increasing production of soybeans in the United States.

U. S. soybeans could play a dominant role in alleviating the protein shortage in developing countries and elsewhere around the world, if soybean meal, flour, protein, and protein concentrates can be successfully used in food products tailored to meet the various nutritional and palatability requirements. Achievement of the maximum share of foreign food markets will require intensive research to acquire more basic information on components that affect nutritional quality, flavor, and other important characteristics of soybean food products. In addition, better knowledge of the effects of processing on these components is needed.

Soybean oil, now the major edible oil of the United States, is the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil. To increase opportunities for foreign utilization of soybean oil, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value; to determine the extent to which minor constituents influence flavor and other properties of the oil; and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. A broad program of basic and applied research is required to achieve the objective.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing, long-range program involving analytical, organic, and physical chemists, biochemists, and chemical engineers engaged in basic and applied research on edible uses of soybean oil, meal, and protein. Food technologists are also required by the program in connection with formulation and organoleptic evaluation of edible products. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components, and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal, and organoleptic stability. Objectives of research on soybean meal and protein are to obtain basic information on the characterization of proteins, enzymes, and other components of soybean meal and to apply the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein in food products for foreign consumption.

The Federal scientific effort for research on utilization of soybeans in foods totals 30.3 scientist man-years. Of this number, 7.0 are devoted to chemical composition and physical properties; 14.0 to flavor; 2.4 to color, texture and other quality factors; .5 to microbiology and toxicology; and 6.4 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (5.6 scientist man-years) includes basic studies on the phenomenon of heat-gelation of alcohol-washed soybean protein and investigation of mass spectroscopy in elucidation of the chemical and molecular structure of glyceride oils and their derivatives. Grants (1.4 scientist man-years) have been made to the University of Minnesota, Minneapolis, Minnesota, for a study of antinutritional factors in soybeans; and to the University of Illinois, Urbana, Illinois, for physiological studies on gastrointestinal effects of soybean protein foods.

Research at Peoria, Illinois, on flavor (12.8 scientist man-years) emphasizes basic and applied studies on selective hydrogenation as a means of stabilizing soybean oil by removal of linolenate. The work includes chemical, physical, and organoleptic evaluation of edible soybean oil products. A research contract (.8 scientist man-year*) is in effect at Rutgers, The State University, New Brunswick, New Jersey, for basic studies on heterogeneous catalysts. In addition, a contract is in effect with the University of Illinois for studies of the mechanism of homogeneous catalysts of hydrogenation by organometallic catalysts. A portion of this effort (.4 scientist man-year) is allocated to research on food uses of soybean oil. During the year, the University of Illinois, Urbana, Illinois, completed a research contract covering basic research on homogeneous catalysts.

Research at Peoria, Illinois, on color, texture and other quality factors (2.1 scientist man-years) is devoted to basic studies of the influence of minor constituents of the soybean on the flavor and other edible qualities of soybean protein food products. A research contract (.3 scientist man-year) at the University of Illinois, Urbana, Illinois, provides for investigation of factors possibly present in soybeans that could cause digestive disturbances.

Research on microbiology and toxicology conducted at Peoria, Illinois, (.5 scientist man-year*) is concerned with a survey to estimate the incidence of aflatoxin in commercial samples of soybeans.

Research at Peoria, Illinois, on technology--process and product development (6.4 scientist man-years) includes engineering studies on production of full-fat soy flour by processes suitable for use in developing countries and

*Work covers more than one commodity; only effort allocated to soybeans is included in total.

on pilot-plant-scale hydrogenation of soybean oil with new selective heterogeneous catalysts. The work on full-fat soy flour is supported by the Agency for International Development and involves cooperation with UNICEF.

The Department also sponsors research on food utilization of soybeans conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the University of Tokyo, Tokyo, Japan, for studies on soybean sterols in defatted meal (5 years, 1963-1968); to Kagawa University, Takamatsu, Japan, for investigations of enzymatic hydrolysis of soybean oligosaccharides (3 years, 1966-1969); and to the Weizmann Institute of Science, Rehovot, Israel, for investigations on glycoproteins of soybean meal (5 years, 1967-1972). During the reporting period, research was completed on complexes between soybean protein and other components of the meal at the Weizmann Institute of Science, Rehovot, Israel.

Research on flavor is conducted under a grant to the University of Tokyo, Tokyo, Japan, for investigations on the flavor components of enzymatically or chemically modified soybean meal and proteins (4 years, 1964-1968). During the year, studies were completed at the University of Granada, Granada, Spain, on the effect of processing on frying quality of soybean oil; at Toyo University, Kawagoe, Saitama-ken, Japan, for research on hydrogenation of soybean oil; and at Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on certain metal chelate compounds as catalysts for selective hydrogenation of soybean oil.

Research on color, texture and other quality factors involves a grant to Sugiyama Chemical Research Institute, Tokyo, Japan, for basic studies on the color reversion of soybean oil (4 years, 1964-1968).

Research on microbiology and toxicology involves grants to the Japan Tofu Association, Tokyo, Japan, for studies on the use of U. S. soybeans for making tofu (5 years, 1963-1968); to Institute of Chemistry, Academia Sinica, Taipei, Taiwan, for investigation on preparing Chinese cheese from soybeans (5 years, 1963-1968); to Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, for studies on improved strains of Saccharomyces rouxii for making shoyu and miso (5 years, 1963-1968); to Japan Shoyu Research Institute, Tokyo, Japan, for comparative evaluation of U. S. and Japanese soybeans and processing methods for making soy sauce (3 years, 1965-1968); and to Tokyo University of Education, Tokyo, Japan, for basic studies on development of foods from enzymatically treated soybean protein concentrates (3 years, 1965-1968). During the year, research was completed at the Central Miso Institute, Tokyo, Japan, on miso made from dehulled soybean grits, and at the Bar-Ilan University, Ramat Gan, Israel, on miso-type food products for use in Israel.

Research on technology--process and product development involves a grant to the University of Tokyo, Tokyo, Japan, for evaluation of U. S. soybeans and processing for the manufacture of dried tofu (3 years, 1966-1969). During the reporting period, research was completed at the Israel Institute of Technology, Haifa, Israel, for evaluation of the quality of isolated soybean protein for use in Israeli foods.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 2.6 scientist man-years is devoted to research on food uses of soybeans.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Mass spectroscopy of glyceride oils and derivatives. Mass spectroscopic analyses of products of the incubation of linoleic acid in the presence of lipoxidase and of water and oxygen, one of which was labelled with heavy oxygen, proved that oxygen incorporated into the products comes from gaseous oxygen. A computer program was written that permits automatic direct conversion of the output of the mass spectrometer, obtained as a punched tape, to the final graphical plot of normalized intensity against mass number. By use of a one-piece all-glass apparatus for collection of volatile constituents, which eliminates any possibility of material being emitted from or absorbed by grease in joints and stopcocks, the presence of hydrocarbons of low molecular weight in autoxidized soybean oil was confirmed. A new field ionization source has proved useful in identification of compounds, such as alcohols and highly branched hydrocarbons, which do not form good molecular ions. An example is methyl 18-hydroxystearate, which has a normal mass spectrum different from that expected for a long-chain alcohol.

2. Basic studies on soybean protein. Hydrolysis of material extracted from isolated soybean protein by 86 percent ethanol yielded sugars identified as glucose, galactose, rhamnose, arabinose, xylose, and glucuronic acid. These sugars were also identified in the hydrolysis products of a presumed saponin fraction. Allantoinase was isolated from soybean meal, and evidence was obtained that allantoinase may be more stable to heat than urease. Methods were devised for recovering 11S protein from aqueous extracts of soybean meal by cold precipitation. Increasing temperature of extraction from 25° to 40° C. resulted in increased amounts of 11S in the extracts and in nearly twofold increase in yield of 11S precipitates on cooling. By use of known procedures for separating other proteins, the 2S, 7S, and 15S impurities, an 11S protein fraction was obtained in 92-95 percent purity. Remaining 5-8 percent (the 7S impurity) was separated by gel filtration to yield an 11S protein homogeneous by ultracentrifugation. Soybean proteins can be solubilized at pH 4.5 by using salt solutions. Maximum extraction occurred at 0.7N with NaCl and at 0.3N with $CaCl_2$.

3. Minor constituents of soybeans. These studies are being conducted by several foreign institutions under PL 480 grants. In research completed at the Weizmann Institute of Science, Rehovot, Israel, enzymatic degradation studies of the glycopeptide isolated from soybean hemagglutinin indicate that mannose residues are alpha-linked and N-acetyl glucosamine residues are beta-linked. Periodate oxidation studies were initiated to elucidate the structure of the glycopeptide. Four distinct hemagglutinins were isolated from soybean meal by diethylaminoethyl cellulose chromatography. The most abundant hemagglutinin is identical with the previously described purified hemagglutinin. All four hemagglutinins are similar in amino acid composition and are glycoproteins containing mannose and glucosamine.

At the University of Tokyo, Tokyo, Japan, confirmatory evidence for acylated steryl glucosides in soybeans was obtained by synthesis of 6-O-palmitoyl-D-glucoside. The synthetic compound and the acylated steryl glucoside fraction isolated from soybeans were identical by infrared analysis and thin-layer chromatography. Differences in melting points, optical rotations, and elementary compositions were observed but appear to result from the mixture of fatty acids (C_{14}-C_{22}) occurring as the acyl group in the material isolated from soybeans. The glucosidic linkages in sterol glucosides and acylated sterol glucosides of soybeans appear to be of the beta form on the basis of their optical rotations when compared with synthetic compounds known to be of the beta configuration. The Florisil column chromatographic procedure for fractionation of soybean sterols has been improved to permit separation into free, glucoside, acylated glucoside, and esterified forms of the sterols. Fourteen U. S. varieties of soybeans were analyzed by the improved procedure. Work is continuing in attempts to remove nonsterol impurities which interfere with colorimetric determination of the different forms of the sterols.

Preliminary results have been obtained on the digestibility of soybean oligosaccharides through in vitro enzymatic studies at Kagawa University, Takamatsu, Japan. Of 17 strains of Escherichia coli grown in the presence of raffinose, only two strains appeared to metabolize the sugar. One of these strains also consumed stachyose. Strains consuming raffinose produced acids causing a drop in pH from 7.0 to 5.4. Examination of the E. coli strains for α-galactosidase activity, as measured by decomposition of melibiose, indicated that the enzyme was primarily intracellular.

In other research at Kagawa University, Takamatsu, Japan, analysis of six U. S. and three Japanese varieties of soybeans showed that hexane-extracted soybean meal averaged 6.4 percent sucrose, 1.3 percent raffinose, 4.8 percent stachyose, and traces of mono- and pentasaccharides. These sugars were shown to be the source of the toasted taste acquired by soybean meal when it is processed for feed. Autoclaving of defatted soybean flakes caused a decrease in total sugar content, an increase in reducing sugars, a decrease in nonreducing sugars, and a decrease in available lysine. It was also shown that soybean oligosaccharides are partially hydrolyzed at pH 4.3,

the isoelectric point of soybean globulins. Work on this project was completed in the past year. Results included a detailed chromatographic analysis of the kinds and amounts of sugars in soybeans.

B. Flavor

1. Selective hydrogenation - homogeneous catalysis. By use of radioactive tracers, evidence was obtained that in hydrogenation of polyunsaturated fatty esters with $Fe(CO)_5$ as catalyst, the diene-$Fe(CO)_3$ complex is an essential intermediate and accounts for the high selectivity observed. On the other hand, methyl oleate was readily hydrogenated with $Fe(CO)_5$ as catalyst with no formation of complex. In mixtures, diene hydrogenation predominates. These findings were confirmed by analog simulation of the kinetics. If complex alone is used as catalyst, simulation showed that the direct reduction path from linoleate to monoene (i.e., not involving complex as intermediate) becomes important. Hydroformylation (oxo reaction) of vegetable oils and unsaturated fatty esters carried out with hydrogen, carbon monoxide, and dicobalt octacarbonyl resulted in addition of -CHO or $-CH_2OH$ groups, hydrogenation of polyenes to monoenes, migration and isomerization of the residual double bond. The isomerized bond is found at various positions along the fatty acid chain with a significant amount in the terminal position. Such isomerization is difficult to achieve by any other means. Conversion of fatty esters to oxo products varied from 42 to 89 percent.

Study of catalytic activity of Pt-Sn complexes under a contract with the University of Illinois showed that solvents displaying minimum capacity to coordinate with the metal were the most favorable for use in homogeneous hydrogenation. Complexes of Pt, Pd, and Ni with $(C_6H_5)_3P$ or $(C_6H_5)_3As$ were effective catalysts for hydrogenation of methyl cis,cis-9,15-octadecadienoate to produce mostly trans monoenes. This result indicates that the double bonds migrated to become conjugated and were then hydrogenated. Complexes of the type MX_2 $(QPhn)_2$, (M = Pt or Pd, X = halide, Q = phosphine or arsine when n = 3 and S or Se when n = 2, Ph = phenyl) catalyze the hydrogenation of polyolefins only in the presence of stannous chloride. Complexes of type PtX_2-(bidentate diene) also catalyze the hydrogenation of polyolefins. The product is the monoene in all hydrogenations studied. Kinetics of homogeneous isomerization of 1,5-cyclooctadiene catalyzed by $PtCl_2(PPh_3)_2$ plus $SnCl_2$ suggests that isomerization occurs in two consecutive reversible first-order reactions. $SnCl_2$ is essential for the isomerization process. It is suggested that it activates the platinum catalyst by being coordinated to it through the ligand $SnCl_3^-$.

During the year, research was completed by the Experiment Station for the Fats and Oils Industry, Milan, Italy. The final report has not yet been received.

2. Selective hydrogenation - heterogeneous catalysis. Partial hydrogenation of soybean oil using commercial copper-chromium catalyst was successfully scaled up to 15-gallon batches in the pilot plant. Linolenate-linoleate selectivity ratios of about 12 were achieved. During hydrogenation, linoleate content remains essentially constant while linolenate content is reduced to about 1 percent. It was found that heat treatment alone was effective for activating commercial copper-chromium catalysts so they would perform satisfactorily at comparatively low pressures (ca.30 p.s.i.) required for selective hydrogenation. Furthermore, active catalysts could be used five successive times without reactivation if they were kept slurried in soybean oil between uses. Cu content of oil increased considerably during hydrogenation. If the oil was bleached, deodorized, treated with citric acid (0.1%), and filtered, copper was reduced to about the level in the original unhydrogenated oil (0.02 p.p.m.).

Laboratory studies on the use of copper-chromium catalysts to selectively hydrogenate soybean oil showed that linolenate could be reduced to less than 1 percent with either laboratory or commercially prepared catalysts. Linolenate selectivity ratios (K_{Le}/K_{Lo}) ranged from 6 to 13. No stearate was formed. Trans isomers produced varied from 7 to 12 percent. Except in the trans isomers, most (90% or more) of the double bonds remaining after hydrogenation were located in their original position. Trans bonds showed extensive migration. A rapid, accurate procedure was developed for gas chromatographic determinations of the fatty acid composition of 2-3 microliter samples of vegetable oils. The micro-technique combines transesterification and sample injection into a single operation. Fatty acid synthesis in green soybeans was studied by incubating slices of freshly picked beans with acetate-1-C^{14} and then measuring the isotopic carbon incorporated into the fatty acids as a function of time. After 2 hours, the specific activity of the polar lipid fraction was 20 times that of neutral lipids. Results indicate that initial desaturation occurs on a 12- or 14-carbon chain, followed by chain elongation to hexadecenoic and oleic acids.

Under a contract with Rutgers University, basic studies on hydrogenation catalysts from leached vermiculites and on infrared absorption of fatty methyl esters adsorbed on catalytic surfaces are essentially completed, and results are being interpreted.

Under a PL 480 grant, research at Toyo University, Kawagoe, Saitama-ken, Japan, shows that copper-nickel-kieselghur catalysts are more active for hydrogenation when activated by dry rather than wet procedures. In the absence of oil or other liquids, optimum activity appears to be achieved when the catalyst is reduced at 190-195° C. for 20 minutes. Manganese enhances activity of copper-chromium or copper-nickel catalysts. Selectivity of copper-nickel for linolenate is not increased by manganese. Isoamyl gallate and citric acid improve resistance to oxidation at 62-63° C. but not at 200° C. of partially hydrogenated soybean oil (iodine value 118). In studies on continuous hydrogenation, the designed equipment failed to

perform as well as batch operations in a larger vessel. A larger scale continuous hydrogenator is now being designed.

3. Evaluation of edible soybean oil products. Studies on evaluation of edible soybean oil confirmed previous findings that residual traces of metal in oils hydrogenated with copper-chromium catalysts adversely affected stability and quality. Preliminary tests showed that heating such oils with citric acid gave products having substantially improved flavor and stability. For the satisfactory quantitative determination of trace metals in oil, ashing appears to be the most effective means of concentrating trace metals in samples for analysis. The amount of volatile hydrocarbons in edible soybean and cottonseed oils shows high correlation with flavor score and peroxide content of the oils. Lipoxidase studies basic to oil processing have established that some enzymes are very specific for the pentadiene system and induce oxidation only on the 13th carbon of linoleic acid. This added oxygen is derived from air and not from the aqueous reaction system. Chromatography showed that the oxidation products contained both hydroperoxide (60%) and a mixture (40%) of oxidative dimer and other polar compounds. Commercial enzyme preparations varied widely in specificity and activity.

At the University of Granada, Granada, Spain, vessels of glass, aluminum, and steel were studied to determine their effect on the stability of fats when used five times for deep-fat frying. Oils tested were pure olive oil and soybean oil refined in Spain. Hake, a fish similar to cod, was used as the test material. Taste panel results indicated that the third and fifth fryings of hake in soybean oil in glass or steel vessels were of better quality. The fifth fry of hake in pure olive oil in an aluminum vessel was best. The influence of antioxidants is being studied and it has been determined that with pure olive oil the optimum quantity of antioxidant that can be added to pure olive oil is 0.6 percent when frying at 180° C. (356° F.) in glass with 1 liter of oil and 1 kilogram of potatoes. Phytic acid and citric acid are being studied as metal inactivators both alone and with antioxidants. These studies, conducted under a PL 480 grant, were completed during the year.

4. Flavor components of soybean meal and protein. The flavor components of defatted soy flakes (0.14% fat content--official analysis) have been found in the bound lipids and in a nonlipid fraction. The flakes were found to contain about 4 p.p.m. of volatile carbonyl compounds. The main component is hexanal, but acetone, acetaldehyde, and an unidentified dienal were also isolated. The bound lipid fraction of either raw or toasted soybean flakes had an astringent taste and a hydrocarbon-like flavor. The nonlipid fraction was bitter. It was observed that the "beany flavor" of raw defatted meal disappeared very rapidly during extraction with alcohol. Extraction of lipids of laboratory-prepared defatted soy flakes with an azeotropic mixture of a hexane-absolute ethanol yielded nearly 3 percent lipids. Composition of crude lipid was 2.2 percent P, 1.05 percent N, and

9 percent carbohydrate. Phospholipids, triglycerides, and sterol derivatives, in decreasing order, were the major constituents. Fractionation of the crude lipids into distinct classes gave phospholipids having an oily, waxy taste and none of the flavor of soy flour. A flavor fraction isolated from the crude lipid extract had a strong hydrocarbon flavor, intense aftertaste and a throat-catching sensation associated with objectionable flavor of the bound lipids.

Under a PL 480 grant to the University of Tokyo, Tokyo, Japan, the following proteolytic enzymes were used to modify the flavor of isolated soybean protein: pepsin, morsine, coronase, and rapidase (acid proteases); prozyme, thermoase, and takadiastase (neutral proteases); and alkaline proteinase of Bacillus subtilis. Coronase and pepsin gave a bitter product. Pronase, bioprase, and alkaline protease of Bacillus subtilis gave a bitter and astringent product. Morsine, rapidase, takadiastase-SS and thermoase gave better flavored products because the bitter and astringent flavors were substantially reduced. Morsine (2 hours' incubation) was judged to give the best flavored products. Combinations of enzymes were no better than morsine-treated products. Takadiastase-SS gave the highest amino-nitrogen values. Hydrolysis with morsine and fractionation by gel filtration and amino acid analyses gave high yields of arginine, leucine, phenylalanine, glutamic acid, lysine, aspartic acid, and tyrosine, trace amounts of cystine and methionine, as well as 13 peptides and 8 unknowns.

C. Color, Texture and Other Quality Factors

1. Flatulence factor of soybeans. In contract research at the University of Illinois, it was shown that fermentation of soybean oligosaccharides in the ileum and colon is apparently one cause for flatulence from soybeans. In vivo studies with anesthesized dogs showed that syringic acid (major phenolic acid in soybean flour) inhibits flatulence in the colon and ileum, whereas genistin has little effect. In vivo production of flatus from tempeh is much less than that from equivalent amounts of soybean flour. Removal of low molecular weight constituents by soaking and cooking effect this result. Demonstration that intestinal microflora of dogs and humans produce gas from soybean oligosaccharides provides evidence that substrate is a primary factor in flatulence. Chromatographic experiments show that E. coli and anaerobic microflora of the dog intestine degrade the soybean oligosaccharides sequentially to monosaccharides followed by the production of gas and concomitant disappearance of carbohydrate components. Similar results were obtained with soy flour. Improved in vitro assay procedures for measuring gas production reveal that with proper control of cultures and media, both anaerobic and aerobic bacteria produce gas from carbohydrates.

In studies under a PL 480 grant to Sugiyama Chemical Research Institute, Tokyo, Japan, it has been shown that the tocopherol content of extracted crude soybean oil decreased to less than 30 percent of the normal value as the moisture content of the beans was increased to 18-20 percent

(coefficient of correlation 0.93). Soybean salad oil obtained from high moisture beans rapidly exhibited the color reversion phenomena after short periods of storage. The quantity of tocored (an oxidation product of tocopherol) in crude soybean is at a maximum when the moisture level of extracted beans is at 18 percent. Refining of the crude oil removed the greater part of the tocored but about 30 percent of a precursor or a "colorless derivative" of tocored remained in the oil. The conversion of this "colorless derivative" to tocored is reportedly the cause of color reversion in stored soybean salad oil. Tests show that the tocopherol content of salad oil before and after color reversion remains the same. No color problem develops when beans of less than 12 percent moisture are processed. Drying of the rolled flakes is not effective. Distribution of tocopherol within various fractions of soybean cotyledons and hypocotyls has been determined on whole beans and for germinated seeds. Current studies deal with the interconversion of tocored and its colorless derivatives.

D. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of soybeans in foods. Results are reported under "Corn Utilization - Feed," subheading B-1.

2. U. S. soybeans for making tofu. Under a PL 480 grant at the Japan Shoyu Research Institute, Tokyo, Japan, conditions for use in the experimental studies on dried tofu, sometimes called frozen tofu or kori-tofu, have been established. The preparative scheme and conditions for each step, the conditions for study of the browning reaction of stored kori-tofu, and the preservation of the swelling capacity of stored kori have been outlined. Selected varieties have been shipped to Japan under another project and they will be tested, in part, for kori-tofu.

Previous studies by the Japan Tofu Association, Tokyo, Japan, have shown that Hawkeye is among the best, if not the best, of varieties for making fresh tofu. Selected and new varieties have been gathered and shipped to Japan for direct comparison with Hawkeye. The protein in this variety has been shown to coagulate slower than most of the proteins of other varieties. Also, a softer and more gelatinous tofu results. Varieties differ in the range of added coagulant that gives a good product. Hawkeye has a wide range. The relation of phytic acid to the coagulation is now under investigation. In calcium-precipitated protein, the phosphorus-nitrogen ratio is higher than in "sodium"-precipitated protein. Thus, phytic acid content does appear to be related to coagulation of tofu. These studies are being conducted under a PL 480 grant.

Research at the Tokyo University of Education, Tokyo, Japan, conducted under a PL 480 grant, shows that trypsin inhibitors in soybean protein may affect the development of new foods from enzymatically treated soybean

concentrates. Water-extracted soybean protein from Hawkeye soybeans was successfully fractionated into five reproducible fractions by gel filtration techniques with Sephadex G-200. The trypsin inhibitor was concentrated in the fourth fraction and the fifth was devoid of protein. Each of the four protein fractions show different ultracentrifugal patterns. Fraction four had a considerably higher percentage of sulfur-containing amino acids. From fraction four, four trypsin inhibitive fractions have been isolated which probably correspond to those found at NU.

3. Studies on miso and shoyu. These studies are being conducted by several foreign institutions under PL 480 grants. At the Japan Shoyu Research Institute, Tokyo, Japan, work is continuing on the use of soybean grits, Japanese and American soybean meal, and alcohol-washed meal as starting material for soy sauce--shoyu. In pilot studies based on procedures developed at the Sendai Test Plant, seven of eleven plants encountered formation of clods in the steaming and cooking of grits for shoyu. American defatted soybean meal could be fermented by the usual procedure, but it is a difficult raw material to use. Further studies will be undertaken to find better methods of treatment. Initial sample of alcohol-washed soybean meal was damaged and a second shipment is being ordered.

Successful matings of Saccharomyces rouxii have been found and the conditions for making mating established at the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan. This now permits hybrids to be made. Several diploid strains thus far studied show growth in an 18-percent NaCl medium. At the same time, studies on flavor components produced by S. rouxii were investigated and one of the major components is β-phenethylalcohol. This is also formed by the hybrids. Two other important flavor compounds in shoyu produced by yeast are p-ethylguaiacol and p-ethylphenol. However, these compounds in shoyu are made by species of Torulopsis rather than S. rouxii.

Evaluation of miso samples at the Central Miso Institute, Tokyo, Japan, prepared with Aspergillus oryzae by fermentation of defatted soybean flakes and various cereals, when fed to weanling rats, showed that miso protein cannot sustain normal growth and development. This is due in part to the high sodium chloride content of the diets containing miso. Determination of the free amino acids in miso reveals the absence of methionine; but supplementation of the rat diet with l-methionine failed to correct the imbalance of miso protein as evaluated by its protein efficiency ratio. Preliminary results suggest that possibly threonine might be limiting. The amounts of three vitamins, thiamine, riboflavin, and niacin, in the finished preparations were found to be from 65-84 percent of the vitamins in the starting materials. However, none of these are limiting factors because diets were fortified with each of them. The cause of part of the poor nutritional value is, as yet, unknown.

In other research at the Central Miso Institute, it was found that concentrates of the water-soluble byproducts of miso production from soybean grits can be used to grow Aspergillus oryzae as a source of amylase and proteinase used in the anerobic stage of miso fermentation. Economic benefits might be realized from such use of these water-soluble materials which represent as much as 10 percent of the dry soybeans. Maximum proteinase activity occurred in 4 days and maximum amylase activity peaked at 6 days. During a 3-day fermentation the total sugar decreased from 2.6 percent to 0.2 percent. Testing of varieties and strains of soybeans for making miso indicate the following varieties are best: Kanrich, M-1, Mandarin, Traverse, and breeding strains AX-80-39 and AX-84-100.

4. Chinese cheese (sufu). Under a PL 480 grant to the Institute of Chemistry, Academia Sinica, Taipei, Taiwan, studies on the extracellular enzymes of Actinomucor elegans, the principal fungus used in the preparation of soybean cheese, have demonstrated the presence of two proteolytic enzymes--one resembling trypsin and the other chymotrypsin. These were demonstrated on a synthetic medium. Investigations also demonstrated the presence of dipeptidases. Additional studies indicate that Actinomucor, as well as several other molds used in the Chinese cheese fermentation, produce a phospholipase. Substitutes for calcium sulfate for coagulating soybean protein were investigated. When canning was used as a means of preserving Chinese cheese, a very fine, smooth, better textured and tasting product could be made. Seasoning of the canned material involved the use of propionic acid, garlic and Kaoliang wine. Considerable study was devoted to the chemistry of the cell wall of the fungus which becomes part of the cheese. Seven amino acids (aspartic, glutamic, threonine, glycine, alanine, valine, leucine, and/or isoleucine) were found, as well as glucose and glucosamine.

E. Technology--Process and Product Development

1. Full-fat soybean flour. In the village or hand process for making full-fat soy flour, microbiological studies show that care is needed during the drying step to obtain a product having a low bacteria count. Drying in the open air and in direct sunlight had an antimicrobial effect and was superior to indoor drying. Total drying time should be limited to 36 hours. Rat-feeding tests, conducted on several lots of full-fat soy flour produced under varied conditions by the village process, indicated nutritive values and protein retention efficiencies comparing favorably with casein. Refinements in hand-operated flour mills has resulted in a finer flour, giving rise to a smooth, stable soy beverage when homogenized in water. In a second process, the extrusion cooking of soybeans, conditions have been established for the satisfactory operation of the extruder to produce full-fat soy flours. A heat treatment has been developed which deactivates the lipoxidase enzyme to give a stable full-fat soy flour free of rancid odor and flavors. This research is supported by the Agency for International Development.

2. Quality of isolated protein for use in Israeli-type foods. Under a PL 480 grant to the Israel Institute of Technology, Haifa, Israel, studies on the processing variables and pilot-plant conditions that affect yield, color, nutritive value, and organoleptic properties of soy protein isolates (SPI) have been completed. The most rapid and complete extraction of protein from dehulled, defatted soy flour was obtained by extraction with 0.03 N calcium hydroxide solutions, at 55° C., for 30 minutes. SPI was recovered by centrifugation of the precipitated curd at pH 4.6. SPI has excellent functional properties for the spray-drying of bananas and in maintaining banana flavor in storage. The considerable loss of loaf volume of 6 percent SPI in bread was counteracted with the addition of lecithin. Studies on the use of SPI in the manufacture of protein cheeses is being investigated. Hard and soft cheeses have been made. However, difficulties associated with the clotting and ripening processes have been encountered. Rheological and organoleptic properties may vary considerably.

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AREA NO. 12: FLAX UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. In recent years, annual domestic use of linseed oil has ranged from 363 to 394 million pounds in contrast to the postwar high of over 700 million pounds in the early 1950's. This decrease was caused primarily by displacement by synthetic materials capable of better performance, particularly in protective coatings.

To restore the level of use of linseed oil, new or expanded markets are urgently needed. Such markets can be achieved by an adequate program of basic and applied research. Recent studies by Department scientists have resulted in commercial manufacture and sale of linseed emulsion paints for exterior use that are competitive with synthetic resin emulsion paints. Other new products from linseed oil to which Department research is contributing are protective coatings for use in curing fresh concrete and in preventing its deterioration from de-icers and freezing and thawing in winter. These new uses have improved the competitive position of linseed oil in relation to synthetics, but additional research is needed to insure maximum acceptance and consumption of linseed oil in these new markets and to provide still other new or improved products that can maintain and increase its use in all types of protective coatings.

Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry. To furnish a sound basis for chemical modification, a broad program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program involving analytical, organic, and physical chemists and chemical engineers engaged in basic research on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil totals 14.2 scientist man-years. Of this number, .7 is devoted to chemical composition, physical properties and structure; 11.7 to chemical and physical investigations to improve products; .7 to microbiology and fermentation; and 1.1 to technology--process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.7 scientist man-year) involves study of mass spectroscopy for elucidating the chemical and molecular structure of glyceride oils and their derivatives.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (10.8 scientist man-years) emphasizes basic studies on the chemistry of linseed oil and linseed fatty acids with the objective of discovering new reactions and derivatives having potential applications in the chemical and protective coatings industries. The work also includes basic investigations of problems related to development of emulsion paints and coatings from linseed oil and to durability of linseed oil films.

Contract research (.3 scientist man-year) involves the Northern Division's share in support of a cooperative agreement among the Division, North Dakota State University, and the National Flaxseed Processors Association. Research under this agreement is conducted at North Dakota State University and involves preparation and evaluation of linseed oil derivatives for use in improving durability of protective coatings. During the year, contract studies were completed by Stanford Research Institute, Menlo Park, California, on properties and reactions of new vinyl copolymers of linseed oil, and by North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota, on aldehyde oils as components of protective coatings. A grant (.6 scientist man-year) has been made to the University of Illinois at Chicago Circle, Chicago, Illinois, for studies of photochemistry of linseed oil polymers on metal oxide substrates.

Research at Peoria, Illinois, on microbiology and fermentation (.7 scientist man-year) is concerned with exploration of the possibilities of preparing new and useful derivatives by fermentative modification of fatty acids.

Research on technology--process and product development involves research contracts (1.1 scientist man-years*) with Kansas State University, Manhattan, Kansas, for studies on the use of linseed oil as a single coating for both curing and protection of concrete; and with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils. During the year, Archer Daniels Midland Company, Minneapolis, Minnesota, completed contract research involving pilot preparation of various aldehyde oil products needed for developmental investigations.

The Department also sponsors research in this area under grants of PL 480 funds to foreign institutions. Chemical and physical investigations to improve products are conducted under a grant to the Regional Research

*Work covers more than one commodity; only effort allocated to flax is included in total.

Laboratory, Hyderabad, India, for exploratory research on hydroxylation reactions of linseed and safflower oils (5 years, 1963-1968). During the year, research* was completed on studies on stereospecific polymerization of polyunsaturated fatty esters at the Experiment Station for the Fats and Oils Industry, Milan, Italy.

Research on microbiology and fermentation involves a grant to the University of Baroda, Baroda, India, for studies* on production of microbial lipases useful for modifying vegetable oils (5 years, 1965-1970).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.5 scientist man-years is devoted to research on other oilseed crops, including flax.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Mass spectroscopy. Mass spectroscopic investigations of chemical and molecular structure of glyceride oils and their derivatives are relevant to industrial utilization of linseed oil. Results are reported under "Soybean Utilization - Food," subheading A-1.

B. Chemical and Physical Investigations to Improve Products

1. Cyclic fatty acids. Tetrafluoroethylene was successfully reacted with conjugated linseed oil to form adduct in 70-percent yield (based on conjugation). This product will be evaluated in drying oil formulations. Adducts of even-numbered α-olefins (C_6-C_{20}) and methyl octadecadienoate were purified by crystallization and hydrogenated. Products are being characterized. Alkyd resins can be prepared from tetrafluoroethylene adduct of conjugated linoleic acids or conjugated linseed fatty acids that are compatible with selected commercial alkyds. These products are being evaluated as vehicles for paints. Synthesis, purification, and analysis of the glycol formed in the oxidation of the ethylene adduct of conjugated linoleic acid have been satisfactorily accomplished. Diesters of C_{18} saturated cyclic acids from linseed oil have previously shown large (375%) increases in viscosity when subjected to the 400° F. oxidative stability test in the presence of copper. By use of a new inhibitor system, viscosity increase has been reduced to 17 percent.

2. New polymers and derivatives for use in water-soluble and other coatings. Polymers prepared from N,N,bis(2-hydroxyethyl)linseed amide (HELA) dibasic acids and toluene diisocyanate (TDI) and containing ester,

*Effort prorated between linseed and soybean oils.

amide, and/or urethane linkages gave films with a range of hardness values, varied alkali resistance and drying characteristics depending on the polymer composition. Films of polyesteramides of isophthalic acid modified with TDI and baked at 150° and 200° C. for 10-30 minutes reached maximum hardness 1 week after baking, except film baked at 200° C. for 30 minutes which did not increase in hardness. Air-dried films from this polymer achieved equivalent hardness in 1 week. Increasing the amount of HELA in isophthalic polyesteramides resulted in a decrease in molecular weight of polymer and slower drying and softer films. TDI modified polyesteramides (10% excess HELA) from endic anhydride and hydrogenated endic anhydride gave faster air drying and harder films than other polymers of this type prepared to date. New dibasic acids in which the fatty chains are bridged together through 1,2-ethanedithio or 1,6-hexanedithio groups were synthesized from methyl oleate. Films from an H_2S-linseed oil reaction product were hard and alkali resistant when baked 1 hour at 250° C. under CO_2. Copolymers of vinyl tetrahydroxystearate or its di-dioxolane derivative with vinyl chloride gave flexible films.

3. Linseed oil films and emulsions. Tensile properties of unsupported pigmented and unpigmented linseed oil films showed that the unpigmented were much weaker and elongated more than films containing TiO_2 or SnO_2. When water-soaked, the pigmented films retained about two-thirds of their breaking strength at 50 percent R.H. Zinc oxide gave stronger but less extensible films than did the other pigments. When water-soaked, films containing ZnO retained only about one-fifth of the 50 percent R.H. breaking strength. Unpigmented films and those containing TiO_2 and SnO_2 showed little or no swelling when soaked in water, whereas those containing ZnO swelled 19 percent. As little as 1 percent ZnO by volume, either alone or in combination with TiO_2, can be responsible for the undesirable tensile and swelling properties of linseed oil films. There appears to be little difference between M-37 and S-70 linseed oil in this respect. Treatment of ZnO with either organic or inorganic phosphate alters surface properties of this material. Inorganic phosphate causes ZnO to become more water wettable, and organic phosphate treatment results in a more oil wettable pigment. Zinc oxides with either treatment cause less swelling of linseed oil films than do untreated pigments.

Paint formulations were developed that permit the use of cationic emulsifiers in preparing emulsion paints from any paint-grade linseed oil. The paints showed good shelf stability and had good film properties. During the study, it was found that a cationic emulsion paint formulation successful for nonbodied oils was inadequate when bodied oils were used. Changes in emulsifier composition, such as combinations of nonionic and cationic surfactants, resulted in acceptable paint systems from bodied oils. These paints dried rapidly and films prepared on glass were rather insensitive to attack by water. Viscosity-stable cationic emulsion paints were made with only about 1 percent total concentration of emulsifiers and dispersing agents. Films from these paints dry rapidly. A method is suggested for

coating pigments with linseed oil that involves spray drying a slurry of pigment and a solution of linseed oil in organic solvent. The oil-coated pigments can be dispersed in water, but a completely satisfactory surfactant system has not yet been found.

In studies conducted under a cooperative agreement among the Northern Division, the North Dakota State University, and the National Flaxseed Processors Association, preliminary screening revealed that reaction products of linseed oil and mercuric acetate show promise as agents to combat growth of mildew in linseed oil paints. These mercury derivatives are fairly stable to boiling water, copper plate, light, and ultraviolet exposure.

4. Glyceride polymers. Work on the preparation and polymerization of fatty esters and their derivatives was continued under a PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy. Conjugated linolenyl alcohol (t,t,t) and the corresponding bromide were prepared. The latter product was reacted with $TiCl_4$-Et_3Al in hexane for 24 hours but no polymerization occurred. Conjugated isomerized isopropyl linolenate was polymerized with $TiCl_4$-Et_3Al (ratios of 1:1, 1:2, 2:1) at 100° C. for 24 hours. A polymer was produced that showed no evidence of conjugated trans bonds (IR at 10.14 μ). The structure of this product is being studied. Polymerization studies on conjugated isopropyl linoleate and conjugated benzyl linoleate under the conditions described for isopropyl linolenate gave no polymer. Polymerization experiments with conjugated isomerized ethyl linolenyl ether under conditions described for isopropyl linolenate gave a small amount of a solid polymer.

5. Hydroxylation of linseed oil. Five routes are being examined for introduction of monohydroxy functions into safflower and linseed oils--epoxidation, sulfation, autoxidation, bromination and selenation. Presence of a complexing agent during catalytic hydrogenation of a fatty epoxide was found to be an excellent means of preserving residual unsaturation. Cupric nitrate performed as well as silver nitrate as the complexing agent, but palladium, zinc and cuprous chlorides were not effective. By this method hydroxy products can be obtained from unsaturated oils with a gain of 2.1 units of hydroxyl value (HV) per unit of iodine value (IV) lost. Work on the epoxidation route is complete. Sulfation of oleic-rich oils, followed by hydrolysis, yielded products having HV and IV ranging approximately from 50-60. Linoleic-rich oils could not be converted to hydroxy glycerides by this method because excessive side reactions occurred with ease. Autoxidation of safflower oil to a peroxide value of 2,225 and reduction of hydroperoxide gave a conjugated (20%) oil having HV of 80 and an IV of 122. Attempts to achieve initially greater peroxide values were not successful. Use of silver salts for replacement of bromine by hydroxyl in safflower oil brominated with N-bromosuccinimide gave incomplete reaction, product typically showing about 3 percent residual Br and HV 100. Oxidation of methyl oleate with SeO_2 (0.5 mole) gave a product having IV 67 and HV 105.

6. Aldehyde oils and derivatives. Research on preparation of aldehyde oils and derivatives is relevant to industrial utilization of linseed oil. Results are reported under "Soybean Utilization - Industrial Products," subheading B-1.

C. Microbiology and Fermentation

1. Microbial modification of fatty acids. Research to explore possibilities of microbial modification of fatty acids as a means for preparing new and useful derivatives is pertinent to industrial utilization of linseed oil. Results are reported under "Soybean Utilization - Industrial Products," subheading C-1.

D. Technology--Process and Product Development

1. Linseed oil coatings for concrete. Contract research at Kansas State University indicated that although continued difficulty is being encountered in finding meaningful laboratory tests for screening and evaluation of linseed oil coatings for protecting and curing concrete, field tests uniformly reveal superior properties of such coatings. Early field results were so promising that the NU-developed boiled oil emulsion is now being manufactured commercially. Skid-resistance tests of highways coated with linseed antispalling compound showed that dry skid resistance recovered to a value equal to or exceeding that of uncoated concrete within 3 hours after coating. Wet skid resistance also recovered rapidly, but not as fast as the dry. Freeze-thaw durability tests of concrete beams cured with NU boiled linseed oil emulsion show that at 100 cycles the treatment inhibits freeze-thaw deterioration. Freeze-thaw evaluation of concrete made with linseed-oil-treated Florence limestone (coarse aggregate) indicates that concrete made with treated aggregate was much less deteriorated at 20 cycles than the control concrete. However, at 65 cycles all specimens were almost completely deteriorated.

2. Aldehyde oils and derivatives. Engineering studies on preparation of aldehyde oils and derivatives is relevant to industrial utilization of linseed oil. Results are reported under "Soybean Utilization - Industrial Products," subheading D-1.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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*Research supported by PL 480 funds.

AREA NO. 13: NEW CROPS UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential, as well as by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

Research on new crops has already revealed several promising plant sources of new products that should have valuable industrial uses. These products include water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids.

One new crop, crambe, has a seed oil rich in erucic acid, which is currently obtained from imported rapeseed oil. Crambe has recently achieved commercialization and the outlook for it to become an important crop is encouraging.

To find still other desirable new crops, continued screening and characterization research is needed. Evaluation of the potential of new materials discovered requires further work on their physical and chemical properties and reactions and on processing to obtain maximum recovery from source plants.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-range, continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful

components and in detailed characterization and evaluation studies of selected components that have the greatest industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of collecting trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical screening program to identify sources of unusual and potentially useful components such as oils, fibers, and gums. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research.

The Federal scientific effort for research on new crops as sources of industrial products totals 9.2 scientist man-years. Of this number, 6.9 are devoted to chemical composition, physical properties and structure, and 2.3 to technology--process and product development.

Research on chemical composition, physical properties and structure is conducted at Peoria, Illinois, and includes conduct of the program on screening uncultivated plants for new oils, fibers, gums, and other components of potential value to industry; organic chemical characterization of selected components, especially new oils and fatty acids; and studies on properties of new plant fibers. During the year, studies were initiated on the composition of rotenone-containing extracts from Tephrosia vogelii.

Research on technology--process and product development, also conducted at Peoria, Illinois, is concerned with studies on pulping new fiber plants, primarily kenaf, and evaluation of the pulp in paper, structural boards, and related products.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition, physical properties and structure involves grants to the Swedish Seed Association, Svalof, Sweden, to find new erucic acid oilseeds (5 years, 1963-1968); and to the Institute of General Chemistry, Warsaw, Poland, for studies on synthesis of glycerides from erucic and related fatty acids (5 years, 1967-1972). Research was completed during the reporting period for determination of glyceride structure of erucic acid oils at the Institute of General Chemistry, Warsaw, Poland.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 6.2 scientist man-years is devoted to research on industrial and feed uses of miscellaneous and new crops.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Screening for new industrial oils. Screening of seeds for unusual oils and other constituents continues. During the period, 791 samples were received, of which 424 were new species. Screening analyses were performed on 777 samples and 362 oils were analyzed. Oil from Crepis alpina contained 75 percent crepenynic acid and is the richest source of this acid yet found. This acid was also found this period in additional species of Jurinea (13 and 36%) and Picris (46%), and in Lapsana communis (50%) and Crepis aspera (58%). Vernonia anthelmintica (224 samples) grown in 1966 ranged in oil content from 12-27 percent, and vernolic acid in oil from 37-78 percent, about the same as the 1965 crop. A large lot of Satureja hortensis seed from Washington contained 45 percent of oil with IV of 214 and linolenic acid content of 70 percent. Petroselinic acid of oils determined by ozonolysis procedures ranged from 43 percent (Bupleurum croceum) to 78 percent in Petroselinum crispum and 79 percent in Hedera helix. Advances in methodology has greatly increased the speed and effectiveness of screening. These include (1) adaptation of a microozonolysis procedure to achieve facile estimation of petroselinic and oleic acid components of the oils; (2) direct analysis of oils by high temperature programmed GLC; and (3) direct instrumental conversion of GLC data followed by processing with a digital computer which interprets data and prints out results. Cephalotaxus drupacea continues to be of interest in the anti-tumor screening; the alkaloid fraction from the seed was found to be active.

Continued research on single seeds and single plant selections at the Swedish Seed Association, Svalof, Sweden, has provided a maximum value of 66 percent C_{22} acids in seed oil from any of the species under study. Variation in erucic acid content of Brassica carinata oil from single plants is not as great as that found for single seeds, but still suggests probable success in developing high erucic lines. The amount of oxazolidinethione derived from thioglucosides in 15 lines of crambe (10-12 mg./g. dry, fat-free meal containing pericarp) varied little, but, in four lines of B. napus, the variation was great enough (7-11 mg./g. dry, fat-free meal) to encourage further research in breeding for low thioglucoside content. In B. campestris, oxazolidinethione from the thioglucosides ranged from 0-6 mg./g. and volatile isothiocyanates from 5-23 mg./g. Development of B. campestris with no oxazolidinethione or isothiocyanate seems possible. Emphasis will be increased on breeding B. carinata for high erucic acid content and on the search for variation in thioglucoside content of high erucic oil producers. These studies are being conducted under a PL 480 grant.

2. Characterization of seed oils and component fatty acids. A new allenic fatty acid (-)-5,6-trans-16-octadecatrienoic acid was isolated from Lamium purpureum seed oil and characterized. Etherification of the hydroxyl group

was found to occur where methyl 13-hydroxy-cis-9,trans-11-octadecadienoate (derived from Xeranthemum annuum seed oil) was treated with 0.1 N methanolic H_2SO_4. The trihydroxy acids of seed oils of three species of Chamaepeuce have been characterized. Cardamine impatiens seed oil has been shown to contain about 40 percent of the glycerides which have monoacetates of dihydroxy acids (mostly C_{22} and C_{24}) attached at α-positions of the glycerides and the β-position occupied primarily by C_{18} unsaturated acids. Two unusual triglycerides make up ca. 85 percent of Chamaepeuce afra seed oil. Each contains one of the trihydroxy C_{18} acids described previously; but in one fraction, each mole of trihydroxy acid is esterified with a mole of C_{18} unsaturated acid and a mole of C_{10}, C_{12} or C_{16} saturated acid. The other component has each mole of the trihydroxy acid moiety esterified with a mole of C_{16} saturated or C_{18} unsaturated acid and a mole of acetic acid. Fatty acids containing isolated double bonds in the 5-position were found in seed oils of other Ranunculaceae. Hence, the discovery that Caltha palustris seed oil contains three such acids suggests that this type of unsaturation may be a characteristic of this family. Analyses of kenaf juice obtained from plants grown at Peoria shows presence of about 20 percent reducing sugars, 2.7-3.8 percent nitrogen (85-95% appears to be non-protein nitrogen), and 20-30 percent ash in juice solids. Jarilla chocola produces edible fruits containing a protease resembling papain in that it responds to sulfhydryl activators.

Under a PL 480 grant to the Institute of General Chemistry, Warsaw, Poland, six seed oils high in esterified erucic acid (30-79%) have been analyzed for component triglycerides. The oils represent the genera Tropaeolum, Iberis, Alliaria, Peltaria, Raphanus, and Crambe. Partial (selective) enzymatic hydrolysis of the oils was followed by separation of the 2-monoglycerides. Gas-liquid chromatographic analysis of fatty acid esters derived from whole oil as well as from the monoglyceride fraction provided data to allow calculation of the positions of fatty acids in the original triglycerides. Tropaeolum oil (79% erucic acid) contains 46 percent trierucin, but does not show the expected preference of erucic acid for the 1,3-positions; the other five oils do show such a preference. Four seed oils from the genera Tropaeolum, Crambe, Eryssimum, and Lobularia are being studied by another, more sophisticated method for triglyceride analysis. These oils have been separated into fractions containing a given number of double bonds per triglyceride. The total fatty acid composition of the individual triglycerides has been calculated, but the positions of the acids on glycerol is not yet known.

3. Rotenoid determinations in Tephrosia vogelii. A qualitative procedure was developed for examination of Tephrosia vogelii extracts by thin-layer chromatography (TLC). By use of this procedure, two rotenoids not previously known to be produced by T. vogelii were found in such extracts in addition to rotenone and deguelin. Stable derivatives of rotenone and deguelin were discovered and used as a basis of development of a quantitative procedure for their determination by GLC. A second method has been

developed for quantitatively determining the amount of rotenone and deguelin in T. vogelii extracts by TLC. Treatment of developed plates with nitric acid vapor and then ammonia results in a Tollens reaction between the degraded rotenoids and silver ions in the support, and resulting brown spots can be measured with a densitometer. Analysis of a large group of samples shows good agreement in results from both of the new methods, GLC and TLC. For three of the breeding lines of T. vogelii, a marked variation of rotenoid content with area of growth and method of handling collected material was found. Two other lines produced deguelin in the above-ground parts, but virtually no rotenone.

4. Screening for new pulp fiber plants. In preparation of pulps from five Georgia-grown French "paper" sorghums, less chemical consumption was observed than with kenaf. Yield of pulp from one variety was greater than from either green or frost-killed kenaf at essentially the same degree of pulping, but freeness was less. The sorghum pulps developed less bursting and tear strengths and folding endurance than do kenaf pulps.

B. Technology--Process and Product Development

1. Fiber content of kenaf. Useful fibrous material from kenaf appears to be more plentiful per plant when planting density is high (narrow rows and close seeding). The essentially uniform content of bast and core fibers in defoliated stalks permits considerable flexibility in harvesting schedules once a maturity of 120 days has been attained. Crude protein in foliage was 30 percent at 90 days after seeding, and it dropped to 20 percent by frost. In a similar period, protein values for defoliated stalks and bark changed from 10 to 5 percent content. Initial indications are that mineral content is greatest in the foliage and least in defoliated stalks. Promising pulps were obtained from ensiled green kenaf with or without addition of molasses to help control growth of undesired microorganisms. With addition of molasses, chopped Florida-grown green kenaf was preserved under essentially anaerobic conditions for 16 months. Results of sulfate pulping such "silage" and of 4 months' silage from Illinois kenaf are similar, but magnitude of pulp yields reflect influence of greater maturity of kenaf possible in the South--Illinois material yielded 43 percent and Florida material 54 percent. Efficiency of bleaching kenaf chemimechanical pulps was improved but brightness still did not meet requirements for newsprint. Evaluation of frost-killed kenaf and weathering tests being conducted in Georgia indicates some deterioration of cellulose content occurring in baled kenaf if the material is stored at too high a moisture content.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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AREA NO. 14: NEW CROPS UTILIZATION - FEEDS

Problem. The research program on new crops is a long-range effort to find new and profitable crops that would have different end-use patterns from those now grown. By providing a wider range of choices, the availability of new crops should enable farmers to achieve more economic use of their land. A more extended discussion of the problems involved in finding and developing a new crop is given under "New Crops Utilization - Industrial Products."

One of the most promising approaches is to search for plants whose seed oils contain potentially useful fatty acids that either are not now available commercially or must be obtained from foreign sources. However, for a new oilseed crop to achieve maximum utility and economic value, it is desirable to obtain, as a byproduct, a palatable and nutritious meal suitable for animal feeds. Thorough investigation is needed, therefore, to determine the probable utility of new oilseed meals as feeds; to discover the presence of possibly undesirable minor constituents; and to evaluate the prospects for successful processing of the oilseed to oil and acceptable meal.

It is possible that a wild plant, although not a potential oilseed crop, might in itself be an advantageous new source of protein or other nutritionally desirable substance. As a part of the broad program on screening and characterization of new plants for potentially valuable components, appropriate effort is required to insure that such a possibility will not be overlooked.

USDA AND COOPERATIVE PROGRAMS

The Department maintains a continuing but limited program involving one professional analytical chemist who devotes a portion of his time to screening uncultivated plants to find possible sources of new amino acids and proteins and to study of amino acids and proteins of meals obtained from new potential oilseed crops.

The Federal program at Peoria, Illinois, totals .4 scientist man-year, all of which is devoted to chemical composition and physical properties.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 6.2 scientist man-years is devoted to research on industrial and feed uses of miscellaneous and new crops.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Components of autolyzed rapeseed meal. Studies have been made involving the structural characterization of the nitrile mixture obtained from a defatted, autolyzed rapeseed meal (Brassica napus). It has been shown that the unsaturated hydroxy nitrile and isomeric episulfides formed were enantiomers of the corresponding compounds derived similarly from crambe meal.

2. Feeding studies on new seed meals. Vernonia meal fed to rats for 90 days at a diet level of 25 percent caused some growth inhibition but no pathological effects. Adding methionine corrected two-thirds of the growth inhibition. Rats fed Euphorbia meal for 4 weeks grew normally, and no harmful effects appeared. These studies were conducted with the cooperation of the Pharmacology Laboratory at the Western Division.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

AREA NO. 15: CRAMBE UTILIZATION INDUSTRIAL PRODUCTS

Problem. Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already existed for erucic acid as well as for imported rapeseed oil, which formerly was the only source of this acid. However, the greatest impetus to commercialization of crambe was perhaps the discovery that crambe oil performed better than any other known material as a lubricant in continuous casting of steel. This situation emphasizes the importance of finding the most advantageous specific applications that can contribute to utilization of any new crop in its own right.

To insure optimum development of crambe as a new economic crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and made effective. This goal can be reached through a program of basic and applied research that will provide more information on the chemical and physical properties of crambe oil, its component fatty acids, and their chemical derivatives. When promising leads to possible industrial applications are found, product and process development research will be needed to evaluate the potential and to provide facts and data essential for commercial adoption of crambe oil products in new end uses.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical and organic chemists and chemical engineers engaged in basic and applied research on industrial utilization of crambe oil. The objectives of the work are to obtain new information on reactions of crambe oil and its component fatty acids and to use this information to develop new products for use by the chemical and other industries.

The Federal scientific effort for research on industrial utilization of crambe totals 5.5 scientist man-years. Of this number, 4.2 are devoted to chemical and physical investigations to improve products and 1.3 to technology--process and product development.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (4.2 scientist man-years) is concerned with chemical modification of crambe oil and its component fatty acids, especially erucic acid, to obtain chemical intermediates or derivatives having desirable properties for industrial use.

Research on technology--process and product development involves a research contract (1.3 scientist man-years) with Southern Research Institute, Birmingham, Alabama, for studies on preparation and evaluation of polyamide resins derived from crambe oil.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.5 scientist man-years is devoted to research on industrial and feed uses of other oilseed crops, including crambe.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical and Physical Investigations to Improve Products

1. Chemical derivatives from crambe oil. A procedure has been found for convenient separation of vinyl 2-methylpentyl brassylate (VMB) into a fraction free of divinyl brassylate (DVB) and a fraction rich in DVB. VMB as a comonomer imparts appreciable plasticization to poly(vinyl chloride) (PVC), although low-temperature properties of molded pieces containing it are inferior to those containing external plasticizers such as 2-methylpentyl brassylate and 2-ethylhexyl phthalate. DVB-rich VMB gave softer products than pure VMB, but molding and tensile properties were poor. Evaluation of 12 diesters (butyl to decyl), prepared from mixed diacids obtained via ozonolysis of crambe free fatty acids, as plasticizers for PVC showed that the "azela-brassylates" and the brassylates impart almost identical tensile properties to the vinyl resins, have comparable heat and light stability, and have excellent low-temperature properties. The bis(2-methylpentyl) azela-brassylate compares quite favorably with commercial dioctyl sebacate as a low-temperature plasticizer for PVC; in addition, the mixed ester has much better light stability. Five disubstituted amides of acids in selectively hydrogenated crambe oil have been prepared for evaluation as PVC plasticizers. Tetracyano ethylene oxide adducts have been prepared from methyl erucate and methyl brassidate (trans isomer of erucate). Acid-catalyzed methanolysis of the adducts gives nearly quantitative yields of 2,5-dicyano-2,5-dicarbomethoxy-3(11-carbomethoxyundecyl)-4-octyltetrahydrofurans instead of the expected esters. Evidence suggests that the methanolysis reaction is sterically controlled to provide major products with carbomethoxy groups trans to vicinal alkyl groups. Similarly the adducts are hydrolyzed on silica gel to dicyano diamides, but the parent trans-tetracyano derivative hydrolyzes much more slowly than the cis-isomer.

B. Technology--Process and Product Development

1. Polyamide resins from erucic acid. In contract research at Southern Research Institute, procedures were developed for mechanically reducing large chunks of nylon 1313 polymer to obtain the small fragments needed to permit molding and other evaluation studies to proceed. Its melt-flow properties were similar to the commercial nylon 11 and nylon 610. Commercial injection molding of nylon 1313 was conducted with commercial-type

equipment, using several different extruder dies. There was no operating difficulty. Polymer filled the mold well, indicating good flow characteristics. A stabilizer against thermal and oxidative deterioration is desirable to prevent discoloration. When properties of nylon 1313 were compared to those of commercial nylon 11 and nylon 610, the following conclusions were drawn: (1) nylon 1313 appears suitable for a number of commercial products; (2) there were no problems in processing nylon 1313 or any deficiencies in its properties that would restrict its usage; and (3) the lower water absorption of nylon 1313 may be a significant advantage. Small amounts of nylon 13 have been prepared by melt polymerization of 13-aminotridecanoic acid.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Investigations to Improve Products

Chang, S.-P., Miwa, T. K., and Wolff, I. A. 1967. Alkyl vinyl esters of brassylic (tridecanedioic) acid. Polymer Preprints 8(1), pp. 591-601.

Nieschlag, H. J., Tallent, W. H., Wolff, I. A., Palm, W. E.,[1] and Witnauer, L. P.[1] ([1]East. Util. Res. Develop. Div., Philadelphia, Pa.). 1967. Mixed esters of brassylic acid as plasticizers for poly(vinyl chloride). Polym. Eng. Sci. 7(1), pp. 51-54.

Nieschlag, H. J., Wolff, I. A., Manley, T. C.,[1] and Holland, R. J.[1] ([1]Welsbach Corporation, Philadelphia, Pa.). 1967. Brassylic acid from ozonolysis of erucic acid. Ind. Eng. Chem., Prod. Res. Develop. 6(2), pp. 120-123.

Technology--Process and Product Development

Greene, J. L., Jr.,[1] Huffman, E. L.,[1] Burks, R. E., Jr.,[1] Sheehan, W. C.,[1] and Wolff, I. A. ([1]Southern Research Institute, Birmingham, Alabama). 1967. Nylon 1313: Synthesis and polymerization of monomers. J. Polym. Sci., Part A-1, 5(2), pp. 391-394.

AREA NO. 16: CRAMBE UTILIZATION - FEEDS

Problem. The economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals. Crambe, a new and only recently commercialized oilseed crop developed under the new crops research program, yields a meal that, on the basis of amino acid analysis, should be an excellent feed product. However, as is true for other oilseed meals, such as soybean meal, suitable processing is needed to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals. Needed research includes isolation and characterization of components of crambe meal that are important to nutritional value, flavor, and other essential qualities of a feed. The fate of these components during processing must be investigated in order to learn how to preserve desired components and eliminate or minimize the effects of deleterious ones. Finally, engineering studies are required to translate laboratory findings into economical and practical processes for industrial use.

USDA AND COOPERATIVE PROGRAMS

The Department maintains a continuing, long-range program of basic and applied research involving analytical and organic chemists and chemical engineers engaged in study of the components of crambe meal and in development of effective processes for converting crambe seed to oil and palatable, nutritious meal for animal feed.

The Federal scientific effort for research on feed uses of crambe totals 7.6 scientist man-years. Of this number, 5.8 are devoted to chemical composition and physical properties and 1.8 to technology--process and product development.

Research on chemical composition and physical properties is conducted at Peoria, Illinois, and is concerned with studies on components of crambe meal such as enzymes, other nitrogenous components, pigments, flavor principles, etc.

Research on technology--process and product development, also conducted at Peoria, Illinois, is devoted to engineering studies on processing crambe seed to oil and palatable, nutritious meal.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 1.5 scientist man-years is devoted to research on industrial and feed uses of other oilseed crops, including crambe.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Crambe enzymes. Treatment of epi-progoitrin (epi-PG) with ferrous ion in aqueous solution protected from atmospheric oxygen resulted in formation of nitrile (S)-1-cyano-2-hydroxy-3-butene and of a new compound, (S)-3-hydroxy-4-penteno-thionamide. Nitrile and thionamide in about 1:4 weight ratio were formed overnight at room temperature from epi-PG and 6-8 molar equivalent of ferrous ion. During first 7 hours of reaction, rate of production of thionamide was about same as loss of epi-PG; then the epi-PG was no longer detectable by UV absorption although thionamide formation continued, reaching maximum at about 24 hours. The isomeric (R)-thionamide obtained from rapeseed has about the same toxicity to mice as the (S)-thionamide from crambe. In an isolated enzyme system that converts epi-PG to (R)-goitrin, the addition of an amount of Fe^{++} equivalent to the iron content of crambe meal suppresses goitrin formation. Instead of goitrin, the enzymatic product is chiefly (S)-1-cyano-2-hydroxy-3-butene. This evidence suggests a role for Fe^{+2} in nitrile production in intact crambe meals.

2. Conversion products from epi-progoitrin (epi-PG). Continuing studies on toxic factors of crambe meal confirm the toxicity of isolated or concentrated nitriles by feeding tests on rats. Limited LD_{50} tests with mice showed toxicities of 25 to 100 mg./kilo. Liver damage is produced by some component in the nitrile mixture. Nitriles are more likely to be formed during autolysis of crambe meal than is (R)-goitrin. Results of rat feeding tests on a series of laboratory and commercially prepared crambe meals, some with carbonate treatment and some without, offer the following conclusions based on this experiment only: (1) rewetting processed meals is not deleterious and may be slightly beneficial, and (2) meals containing unhydrolyzed epi-progoitrin gave poorest growth response and most severe lesions in the liver and other body organs. However, defatted meal allowed to autolyze contains no epi-progoitrin but is far more toxic than any of the above.

B. Technology--Process and Product Development

1. Processing crambe to oil and meal. Rat feeding tests show that after treatment of crambe meal with soda ash, some growth inhibitory substances remained. Feeding tests on chicks also provided evidence that these meals were not as good as soybean meal for nonruminants. However, meal treated with soda ash continues to give good results in feeding tests with cattle. Weight gains were equal when the protein supplement was either all soybean meal or a 50-50 crambe-soybean meal mixture. When rats were fed autolyzed and nonautolyzed crambe meals at a 30-percent level, neither growth nor organ toxicity correlated with nitrile content of the meals. Residual

epi-progoitrin or goitrin was more directly associated with poor growth and resulted in larger livers, kidneys, and thyroids. Organs of animals fed meals free of epi-progoitrin were normal even though nitrile content was high. Soda ash improved growth when it accomplished destruction of epi-progoitrin.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Austin, F. L., and Gent, C. A. 1967. (S)-3-Hydroxypent-4-enethionamide: A new reaction product of a natural thioglucoside. Chem. Commun. (2), pp. 71-72.

Daxenbichler, M. E., VanEtten, C. H., and Wolff, I. A. 1966. Diastereomeric 1-cyano-2(S)-hydroxy-3,4-epithiobutanes from epi-progoitrin of crambe seed. Chem. Commun. (15), pp. 526-527.

VanEtten, C. H., Daxenbichler, M. E., Peters, J. E., and Tookey, H. L. 1966. Variation in enzymatic degradation products from the major thioglucosides in Crambe abyssinica and Brassica napus seed meals. J. Agr. Food Chem. 14(4), pp. 426-429.

Technology--Process and Product Development

Kirk, L. D., Mustakas, G. C., and Griffin, E. L., Jr. 1966. Crambe seed processing: Improved feed meal by ammoniation. J. Amer. Oil Chem. Soc. 43(9), pp. 550-555.

AREA NO. 17: FORAGE UTILIZATION - FEED (NORTHERN REGION)

Problem. Tall fescue grass is grown extensively in the Southeast, in the Intermountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. The quality of staying green in the winter is a prime factor in its acceptance. Thirty-five to fifty million acres of fescue are grown for forage use in the Southeastern part of the United States alone.

Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks the animal first becomes lame. The peripheral portion of one or more limbs then develops necrosis, and sloughing of the hooves may occur. Occasionally the tail and ears may be affected. Animals become emaciated and frequently die. The disorder is more apt to occur during cool weather than during the summer months. However, even when conditions are not such as to produce the more dramatic symptoms, cattle sometimes perform poorly on fescue forage, a result which may be attributed to subclinical toxicity.

Pastures may become toxic after several years of freedom from toxicity. Serious outbreaks of fescue toxicity occurred during the winter 1963-64 in parts of Kentucky, Illinois, Missouri, Kansas, and Arkansas. Thousands of head were involved, with morbidity ranging from 1 percent to 99 percent of the herds. For example, 42 of 72 head of cattle became lame after 8 days on one pasture in Missouri. In these outbreaks, the toxic pastures were soil bank lands having long grass that was pastured after the advent of cold weather.

Research to determine the cause of toxicity in fescue and to identify the toxic substance(s) is needed as a basic step in developing a solution to the problem of toxic fescue.

USDA AND COOPERATIVE PROGRAMS

At the Northern Division, Peoria, Illinois, the Department has a program of limited scope that involves one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station, which furnishes toxic and non-toxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at the Western Division.

The major part of the Department's research program on forages in maintained at the Western Utilization Research and Development Division, Albany, California.

The Federal program at Peoria, Illinois, totals 1.4 scientist man-years, all of which is devoted to microbiology and toxicology.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.5 scientist man-years is devoted to research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Microbiology and Toxicology

1. Fescue toxicity. The complete structure of γ-acetamido-$\Delta^{\alpha,\beta}$-butenolide (I) isolated from cultures of Fusarium nivale has been established. It was found that (I) has no pronounced antibiotic activity against 14 bacteria, 3 molds, and 1 yeast. A sublethal dose for cattle of the toxic aqueous-alcoholic extract of F. nivale grown on hay lowered the tailskin temperature, but did not produce gangrene. Aqueous-alcoholic extracts of toxic fescue hay were toxic to rabbit skin even though they contained no demonstrable IR bands of (I). Compound (I), present in F. nivale cultures, is not wholly recovered in such extracts. F. nivale also produces a sesquiterpenoid, a toxin identical to (or an enantiomorph of) isovaleroxydiacetoxyscirpenol, isolated by the University of Wisconsin from F. tricinctum. Samples of forage from a toxic fescue pasture have been gathered while the cattle were sick with fescue foot. This presents the first opportunity to examine the microbiology of field forage as it exists while the cattle are still sick.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Microbiology and Toxicology

Yates, S. G., Tookey, H. L., Ellis, J. J., and Burkhardt, H. J.[1] ([1]West. Util. Res. Develop. Div., Albany, California). 1967. Toxic butenolide produced by Fusarium nivale (Fries) Cesati isolated from tall fescue (Festuca arundinacea Schreb.). Tetrahedron Lett. (7), pp. 621-625.

Keyl, A. C.,[1] Lewis, J. C.,[1] Ellis, J. J., Yates, S. G., and Tookey, H. L. ([1]West. Util. Res. Develop. Div., Albany, California). 1967. Toxic fungi isolated from tall fescue. Mycopathol. Mycol. Appl. 31(3-4), pp. 327-331.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev. 2)	Operation and improvement of a culture collection of molds, yeasts, bacteria, and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins, chemicals, polymers, assays, and identifications of importance to the national welfare.	Peoria, Ill.	Yes	1-C-1
N1 1-182 (Rev.)	Studies on protosexual yeasts, their existence, characteristics, and phylogenetic relationships as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	No	
N1 1-195(C)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.*	Lafayette, Ind.	No	
N1 1-196	Chemical investigations on amylomaize selections to guide corn breeders in the development of commercial hybrids containing high-amylose starch for industrial use.*	Peoria, Ill.	No	
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains.*	Baltimore, Md.	No	
N1 1-204	Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.*	Peoria, Ill.	Yes	4-A-1
N1 1-209	Investigations on the applicability and evaluation of chemically modified cereal grain flours and fractions as ingredients, agents, and adhesives in pulp and paperboard products as a basis for increasing industrial use of cereal grains.	Peoria, Ill.	Yes	1-D-1
N1 1-210	Investigations on the preparation of water-dispersible hetero-derivatives of starch to obtain products having a wide range of properties for the production of adhesives, sizings, and other additives for applications in paper and related industries.	Peoria, Ill.	Yes	1-B-5
N1 1-212	Investigations on the production of low-density plastic foams from starch-derived glucosides and related starch derivatives as a basis for increasing the industrial utilization of cereal starches.*	Peoria, Ill.	No	
N1 1-213	Pilot-plant investigations on wheat dry-milling and fractionation methods for producing a wide variety of products for use in foods, feeds, and industrial products.*	Peoria, Ill.	No	
N1 1-214(C)	Engineering studies on the application of pneumatic fluidization to the reactions of wheat flour with hydrogen chloride as a basis for producing sizing agents for paper.*	Ames, Iowa	No	

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
N1 1-215	Investigations of the reaction of dialdehyde starch with casein, soybean protein, soy flour and dried animal blood for the production of improved wood adhesives.	Peoria, Ill.	Yes	1-B-5
N1 1-217	Investigation of methods for producing microbial polysaccharides from cereal grains by continuous fermentation to reduce production costs allowing increased utilization of these potentially useful gums.	Peoria, Ill.	Yes	1-C-5
N1 1-218(C)	Stabilization of vegetative cells of Bacillus popilliae grown on cereal-based media for use as an infecting agent against the Japanese beetle.*	Manhattan, Kans.	No	
N1 1-219(C)	Study of role of enzymes and enzyme activity in the formation of spores of Bacillus popilliae and Bacillus lentimorbus as a basis for the mass production of biological insecticides by fermenting cereal grain.	East Lansing, Mich.	Yes	1-C-4
N1 1-220(C)	The transfer of genetic determinants of sporulation from one microorganism to another, as a basis for applied studies on the fermentative production of spore dusts for the control of Japanese beetle infestations.	Minneapolis, Minn.	Yes	1-C-7
N1 1-222(C)	Studies on the mechanism and kinetics of radiation and ceric ion induced grafting of cereal starches with vinyl-type monomers previously shown in exploratory studies to graft readily and efficiently with promise for new industrial outlets for starch.*	Menlo Park, Calif.	No	
N1 1-223(C)	Development studies on the semi-pilot-plant scale production of cereal grain xanthides and their use and evaluation in making corrugating board and linerboard for corrugated boxes.*	Columbus, Ohio	No	
N1 1-224	Development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.	Peoria, Ill.	Yes	5-B-1
N1 1-225	Investigations on the development of new fermented wheat foods through the use of Oriental-type food molds as a basis for increasing export markets for U. S. wheats.	Peoria, Ill.	Yes	5-B-2
N1 1-227	Investigations on the conversion of cereal xanthates to xanthides in physical forms suitable for use in papermaking.	Peoria, Ill.	Yes	1-D-1
N1 1-229	Investigations on processing methods for wheat to minimize radioactive contamination in milling products.*	Peoria, Ill.	No	
N1 1-230(C)	Investigations on the synthesis of terminal C4-modified maltooligosaccharides for use in studying enzyme modifications of cereal starches.	Carbondale, Ill.	Yes	1-B-2
N1 1-231(C)	Stabilization of beta-carotene in dried mycelium and in extracted form as a contribution to commercialization of beta-carotene produced by fermentation of cereal grain.*	Cambridge, Mass.	No	

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
N1 1-232(C)	Investigations on the vinylation of methyl glucoside by reaction with acetylene and on the properties and reactions of the products as a basis for development of new industrial outlets for cereal grains.	Tucson, Ariz.	No	
N1 1-233(Gr)	Studies on the types and variations of starch granules within the endosperm of genetically different high-amylose corns to provide fundamental information important to the processing and utilization of high-amylose corn.*	Lincoln, Nebr.	No	
N1 1-234(Gr)	Investigations of two-phase submerged fermentation processes as means for increasing yields and/or concentrations of products obtained by fermentation of cereal grains.	Ithaca, N. Y.	Yes	1-C-5
N1 1-235(C)	Investigation of the morphological changes involved in the transition of Bacillus popilliae from vegetative cells to spores for controlling Japanese beetle infestations.	Houston, Tex.	Yes	1-C-4
N1 1-236(C)	Investigation on the isolation and characterization of phenolic pigments of grain sorghum to provide basic information related to the discoloration of milled sorghum and its starch.*	Bloomington, Ind.	No	
N1 1-237	Investigation of the characteristics and classification of microorganisms of the section Dubiorugorhizopus of the genus Rhizopus of the family Mucoraceae, as tools for use in the development of fermentations utilizing cereal crops.	Peoria, Ill.	Yes	1-C-2
N1 1-238(C)	Studies on kernel properties and milling and fractionation characteristics of wheats exhibiting a range of kernel hardness and protein content to provide information basic to the production of a range of products for industrial uses and application in baking.	Lincoln, Nebr.	Yes	5-A-2
N1 1-239(Gr)	Basic investigations on the chemical and molecular structure of amyloglucosidases with emphasis on relationship to enzyme formation and action to provide information applicable to the production and use of these enzymes in the utilization of cereal grains.*	Lincoln, Nebr.	Yes	1-C-3
N1 1-240	New microbial polysaccharides of commercial value produced from cereal grains: Characterization and structural analysis of previously selected polysaccharides and screening for additional polysaccharides with new and broader range of applicability.	Peoria, Ill.	Yes	1-C-5
N1 1-241	Investigations on molecular structure, aggregation, and interactions of wheat gluten proteins and their chemical modifications to provide basic information related to industrial utilization of wheat.*	Peoria, Ill.	Yes	4-A-1
N1 1-242	Chemical transformations of maltose and dextrose to determine differences in reactivity and to produce new compounds of possible industrial use from these cereal starch-derived sugars.	Peoria, Ill.	Yes	1-B-1
N1 1-243	Modification of fermentations by transfer of genetic material in microorganisms.	Peoria, Ill.	Yes	1-C-7

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
N1 1-244	Exploratory studies on the chemical and physical modification of high-amylose corn starches to improve their applications as coatings and sizings for industrial use.	Peoria, Ill.	Yes	1-B-6
N1 1-245(C)	Development of optimal papermaking processes using cereal grain xanthides made from starch, ground whole grain, flour, bran, shorts, and other dry-milled grain products in blends with wood pulp to produce linerboard, corrugating media, and bag papers.	Columbus, Ohio	No	
N1 1-247	Exploratory studies on the chemical synthesis and characterization of crosslinked starch derivatives having potential value as paper additives for improvement of tear, stretch, and moisture stability of paper products and for upgrading properties of boxwood, insulating board, and other structural materials.	Peoria, Ill.	Yes	1-B-5
N1 1-248(C)	Preparation and evaluation of selected starch graft copolymers for industrial use in plastic products and industrial coatings.	Menlo Park, Calif.	Yes	1-D-2
N1 1-250(Gr)	The reaction of vinyl ethers with carbohydrates, especially D-glucose and starch.	Columbus, Ohio	No	
N1 1-251(Gr)	Basic studies on the relation of viscoelastic properties of amylose sheets and films to structure and function of added plasticizers.	Princeton, N. J.	Yes	1-A-3
N1 1-252(Gr)	Basic investigations on the organic chemistry of unsaturated and sulfur-containing carbohydrates to provide a basis for the development of new reactions and derivatives of cereal grain starches and related sugars.	Columbus, Ohio	Yes	1-B-2
N1 1-253 (Rev.)	Studies on the production of mycotoxins by Aspergillus flavus and related molds to provide basic information for processing grain into feeds.	Peoria, Ill.	Yes	3-B-1
N1 1-254(C)	Development of improved methods for preserving microorganisms that cannot be satisfactorily lyophilized for use in the fermentative conversion of cereal grain into industrial products.	Rockville, Md.	Yes	1-C-1
N1 1-255	Investigation of the sporulation of milky disease bacteria in vivo and in vitro as a basis for the development of a fermentation process for the production of a pesticidal agent against the Japanese beetle.	Peoria, Ill.	Yes	1-C-4
N1 1-256	Exploratory studies on the preparation of new and novel products from unmodified cereal starches and thin-boiling starches by graft copolymerization with selected vinyl and acrylic monomers.	Peoria, Ill.	Yes	1-B-3
N1 1-257	Basic studies on the relation of film properties of amylose to molecular organization and structure in order to provide information needed in improving the preparation and properties of films and coatings from amylose and high-amylose starch.	Peoria, Ill.	Yes	1-A-3
N1 1-258(C)	Application of antimetabolites for selecting mutants of Blakeslea trispora to enhance yields of beta-carotene by fermentation of cereal grain and other agricultural products.*	Chicago, Ill.	No	

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
N1 1-259	Exploratory investigations on the conversion of cereal grain carbohydrates to industrial materials through the use of microbial enzyme systems.	Peoria, Ill.	Yes	1-C-3
N1 1-260(C)	Preparation, characterization, and chemical modification of polypeptides derived from cereal proteins to yield products of potential industrial interest as a basis for increasing the utilization of cereal grains.	Chicago, Ill.	Yes	4-B-2
N1 1-261(C)	Establishment of practical conditions for the application of acid-modified flour as a surface-sizing agent for paper on a semi-commercial paper machine.	Berlin, N. H.	Yes	4-D-1
N1 1-262(C)	Evaluation of cyanoethylated modified corn starches for application in paper processes as a basis for the development of expanded markets for cereal grain products.	Kalamazoo, Mich.	Yes	1-D-4
N1 1-263	Study of plant-protective and chemical properties of antibiotics produced by fermentation of cereal-based media with streptomycetes and correlation of taxonomic characteristics of antibiotic-producing microorganisms with antibiotic identities.	Peoria, Ill.	Yes	1-C-6
N1 1-264	Engineering studies on modifications of cereal flours and starches to prepare useful polymeric products and to evaluate the products and processes.	Peoria, Ill.	Yes	1-D-4
N1 1-265(Gr)	Basic investigations on the mechanical and viscoelastic properties of corn kernels as influenced by tempering conditions employed in the dry-milling process.	University Park, Pa.	Yes	2-C-2
N1 1-266	Investigations on the application of NMR spectroscopy to grain constituents and derived products to provide information on chemical and molecular structure pertinent to utilization research.	Peoria, Ill.	Yes	1-A-3
N1 1-267(C)	Investigations on the protein content, amino acid composition, and biological feeding value of hybrid grain sorghums and selected milling products as a foundation for improvements in the utilization of grain sorghum in foods and feeds.	Manhattan, Kans.	Yes	9-A-1
N1 1-268(Gr)	Synthesis and degradation of O-glycosides of hydroxy amino acids that form protein to carbohydrate linkages in glycoproteins to provide basic information on the reactions of carbohydrates in proteinaceous compounds and specific information useful for characterizing cereal glycoproteins.	Milwaukee, Wisc.	Yes	4-A-2
N1 1-269(Gr)	Investigations on the principles of disc electrophoresis as a method for large-scale separation of proteins and enzymes.	Manhattan, Kans.	Yes	1-C-3
N1 1-270(Gr)	Basic studies on heat, mass, and momentum transport in cereal starches and flours to provide information for the design of more economical processes for chemical and physical modifications.	Ames, Iowa	Yes	1-A-3

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
N1 1-271(Gr)	Investigations on amylases from bacteria and on their action patterns and products found on amylolysis of starch and related substrates.	Ames, Iowa	Yes	1-C-3
N1 1-272(Gr)	Basic research on the preparation and characterization of sugars containing carbon-bound nitrogen, phosphorus, and sulfur.	Lafayette, Ind.	Yes	1-B-2
N1 1-273(Gr)	Basic studies on the mechanism and effects of chemical cleavage of disulfide bonds in wheat and corn endosperm proteins to provide information for improving the processing and utilization of cereal grains.	Lafayette, Ind.	Yes	1-A-2
N1 1-274(C)	Exploratory studies on the suitability of starch derivatives as protective colloids and binders for use in water-emulsion paints.	Columbus, Ohio	Yes	1-D-3
N1 1-275(C)	Studies on the physical and chemical factors that govern retention and effectiveness of starch xanthates and xanthides by wood pulp to provide information basic to use of these products in papermaking.	Appleton, Wisc.	Yes	1-B-5
N1 1-276	Exploratory investigations on the enzymatic conversion of cereal starches and glucose to unique glucosides, polyols and isomerization products of glucose as a basis for increasing the industrial utilization of cereal grains.	Peoria, Ill.	Yes	1-C-3
N1 1-277	Study of principles associated with tall fescue which cause toxicity in cattle or limit utilization of the nutrients of this grass.	Peoria, Ill.	Yes	17-A-1
N1 1-278(Gr)	Studies of starch reactions in a dielectric field as a basis for increasing the industrial utilization of cereal starches.	Pittsburgh, Pa.	Yes	1-B-4
N1 1-279(Gr)	Studies on the amination of starch for the purpose of modifying its chemical and physical properties as a basis for expanded utilization.	Columbus, Ohio	No	
N1 1-280	Investigations on the composition and distribution of lipids in the hybrid corn kernel and changes related to storage and artificial drying to provide information basic to the dry- and wet-milling of corn and production of products of maximum value and utility.	Peoria, Ill.	Yes	3-A-2
N1 1-281	Exploration of microbial spores as fermentative agents for converting cereal grains to industrial products.	Peoria, Ill.	Yes	1-C-3
N1 1-282(C)	Investigation of typical Aspergilli that may grow on cereal grains to produce toxins, as a basis for improving the production of wholesome foods and feeds.	Brookings, S. Dak.	Yes	3-B-1
N1 1-283	Studies on copolymerization, addition and replacement reactions of free and potential functional groups of wheat gluten proteins with vinyl monomers and related chemicals as a basis for developing new industrial products from these proteins.	Peoria, Ill.	Yes	4-B-1
N1 1-284	Engineering investigations on improved tempering and degerminating procedures for dry milling corn of 15-20 percent moisture content, and development of a flotation separation process and of dehulling equipment for an improved process for manufacturing dry-milled corn products.	Peoria, Ill.	Yes	2-C-1

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
N1 1-285	Study of microscopic and ultrastructure of wheat grains and flours and of changes induced by enzymes, moisture, and various treatments to obtain information leading to improved processing methods and new products.	Peoria, Ill.	Yes	5-A-1
N1 1-286(Gr)	Fine structure of microbial polysaccharide NRRL B-1973 obtained by fermentation of starch-derived sugars.	Madison, Wisc.	Yes	1-C-5
N1 1-287	Investigations on the dispersion, solubility, and structure of starches from various corn genotypes which contain different proportions of amylose and amylopectin to provide basic information for their utilization.	Peoria, Ill.	Yes	1-A-2
N1 1-288(Gr)	Studies on the mechanism of hydrolysis of cereal starches by enzymes using Bacillus subtilis amylase and sweet potato beta-amylase as model enzyme systems.	Fayetteville, Ark.	Yes	1-C-3
N1 1-289(Gr)	The reaction of diepoxides with corn starch and its derived products.	Tucson, Ariz.	Yes	1-B-2
N1 1-290	Explorations on new dry-milling processes for wheat and wheat fractions to develop methods for producing a variety of products for use in industrial products, feeds, and foods.**	Peoria, Ill.	Yes	5-C-1
N1 1-291(C)	Studies on the use of starch-derived glycol and glycerol glycosides as protective coatings, plastics, and resins.	Minneapolis, Minn.	Yes	1-D-3
N1 1-292(C)	Development studies on the use of starch and starch derivatives for the reinforcement of synthetic and natural rubber products.	Akron, Ohio	Yes	1-B-7
N1 1-293(Gr)	Basic studies on reactions of the helical form of amylose with small molecules.	Tempe, Ariz.	Yes	1-A-2
N1 1-294	Exploration of methods for the preparation of halogen-substituted derivatives of glucose, starch, and related carbohydrates as a basis for the development of new products for industrial use from cereals.	Peoria, Ill.	Yes	1-B-2
N1 1-295	Investigations of the amounts, distribution, amino acid composition, and molecular structure of the different proteins of corn grain to provide basic information needed for improving the milling of corn for food, feed, and industrial uses.	Peoria, Ill.	Yes	3-A-1
N1 1-296	Engineering studies of processing systems for grain sorghum and other small domestic grains and their fractions, to yield new or improved products for food and industrial uses.**	Peoria, Ill.	Yes	8-B-1
N1 1-297(Gr)	Studies on the reactions of carbohydrate esters as a basis for the chemical modification of starch.**	Carbondale, Ill.	No	
N1 1-298(Gr)	Reactions and transformations of serine in proteins and peptides as a basis for chemically modifying cereal proteins.**	Chicago, Ill.	No	
N1 1-299(Gr)	Structure and formation of the carbohydrate moiety of fungal glucohydrolases active on cereal starches.**	Lincoln, Nebr.	No	
N1 1-300(C)	Investigation of dominant factors influencing productivity of polysaccharogenic microorganisms in converting starch to new polymers.**	To be determined	No	

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
N1 1-301(Gr)	Study of branching in starches and selected microbial polysaccharides by physicochemical techniques and of the influence of ionogenic branches on physicochemical properties.**	Commerce, Tex.	No	
N1 1-302(Gr)	Cellular differentiation and physiology of selected molds as a basis for improving the fermentation of cereal grains to commercial products.**	Minneapolis, Minn.	No	
N1 1-303	Investigation on the production of noncellular polyurethane plastic materials containing starch and starch-derived products as a basis for increasing the utilization of cereal starches.**	Peoria, Ill.	No	
N1 1-304	Investigations on the effect of composition and structure of cereal starches and microbial polysaccharides on their rheological properties as a basis for increasing the utilization of cereal grains.**	Peoria, Ill.	No	
N1 1-305	Physicochemical investigations on the molecular properties of electrophoretically and chromatographically separated proteins of grain sorghum, and on their modified forms, as a basis for increased industrial, food, and feed uses.**	Peoria, Ill.	No	
N1 1-306(C)	Investigation of the biochemical properties of variant cultures of Bacillus popilliae to disclose the physiological processes essential for sporulation when grown on a cereal grain medium to produce a biological insecticide for control of Japanese beetle infestations.**	East Lansing, Mich.	No	
N1 1-308	Isolation and characterization of compounds produced by chemical reactions of corn sugars with amino compounds to provide basic information on the color, antioxidant, and flavor-producing reactions of these sugars in processed corn and grain sorghum food products.**	Peoria, Ill.	No	
N1 1-309	Chemical comparison of the molecular structure of isolated wheat gluten proteins as a basis for their increased utilization.**	Peoria, Ill.	No	
N1 1-310(Gr)	Cytology of ascospore formation in yeasts: Relation to genetic analysis designed to alter the kind and amount of metabolites produced.**	Houston, Tex.	No	
N1 1-311(Gr)	Investigation of starch fine structure by analysis of LASER generated scattering patterns.**	Syracuse, N. Y.	No	
N1 1-312(C)	Evaluation and development of selected water-soluble graft copolymers of starch for use as flocculants, thickeners, sizes, and adhesives, in beneficiation of ores and minerals, clarification of waste waters and sewage, and in the production of paper, textiles, and petroleum.**	Minneapolis, Minn.	No	
N1 1-313(Gr)	Studies on the interaction of phytin with proteins in processing corn to give food products of high nutritional value.**	Columbia, Mo.	No	
N1 1-315(Gr)	Utilization of microbial polymers as a coating for root systems of tree seedlings planted in acid spoil land.**	Terre Haute, Ind.	No	

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
CR-NU-1(C)	Investigation of methods for the rapid determination of critical amino acids in cereal grains.**	To be determined	No	
N4 2	Soybean and other oilseed utilization investigations--Northern region.			
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.*	Peoria, Ill.	No	
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.*	Peoria, Ill.	No	
N4 2-88	Basic investigations on the chemical reactions of soybean and linseed oils and their fatty acids with ethylene and other commercially available olefinic compounds to produce new products having potential industrial value.	Peoria, Ill.	Yes	12-B-1
N4 2-90(C)	Investigations on flatus caused by ingestion of soybean foods as related to the development of foreign-type foods to expand export markets.	Urbana, Ill.	Yes	11-C-1
N4 2-93(C)	Basic investigations on heterogeneous catalysts for the selective hydrogenation of linolenate in soybean oil to provide basic information for increased food applications.	New Brunswick, N. J.	Yes	11-B-2
N4 2-94(C)	Investigations on the conversion of aldehyde oils to coating compositions for wood and metal as a basis for developing new industrial products from linseed and soybean oils.*	Fargo, N. Dak.	No	
N4 2-96 (Rev.)	Relation of minor constituents of soybeans to flavor, flatus, and color of soybean protein food products.	Peoria, Ill.	Yes	11-B-4
N4 2-97(C)	Basic studies on the organo complexes of transition metals as homogeneous catalysts for hydrogenation of soybean oil.*	Urbana, Ill.	No	
N4 2-98	Investigations on heat gelation of alcohol-washed soybean protein as a basis for developing new food and industrial uses for this protein.	Peoria, Ill.	Yes	11-A-2
N4 2-99(C)	Studies on the effect of linseed oil coatings on the curing and durability of concrete, and evaluation of selected linseed oil compositions for this potentially new use.	Manhattan, Kans.	Yes	12-D-1
N4 2-100(C)	Investigations on the preparation of copolymers of linseed oil and vinyl monomers suitable for emulsion paints.*	Menlo Park, Calif.	No	
N4 2-101(C)	Investigations on the chemical and physical properties of poly(ester-acetals) and poly(amide-acetals) derived from soybean and linseed oils and of the bonds formed between them and various substrates after crosslinking.	Dedham, Mass.	Yes	10-D-1
N4 2-102	Microbial modification of fatty acids to produce derivative long-chain acids of potential industrial utility.	Peoria, Ill.	Yes	10-C-1

*Discontinued during reporting year.
**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
N4 2-103	Basic studies on the water permeability and water sensitivity of linseed oil films, as a basis for increasing the utilization of linseed oil in paints.	Peoria, Ill.	Yes	12-B-3
N4 2-104	Investigations on the effect of emulsion and oil viscosities, and of particle size in oil-in-water emulsions on the film-forming properties of linseed oil, as a basis for increasing the utilization of linseed oil in paints.	Peoria, Ill.	Yes	12-B-3
N4 2-105(C)	Pilot preparation of materials for developmental investigations on aldehyde oils and related products derived from soybean and linseed oils.*	Minneapolis, Minn.	No	
N4 2-106	Investigation of chemical and molecular structure of vegetable oils and their derivatives from soybeans, flax and other commodities of the Northern Region by mass spectrometry to obtain information pertinent to utilization research.	Peoria, Ill.	Yes	11-A-1
N4 2-107	Investigation of selective heterogeneous catalytic hydrogenation to provide information basic to increasing the edible use of soybean oil.	Peoria, Ill.	Yes	11-B-2
N4 2-108	Basic investigations on homogeneous catalysts for selective hydrogenation of soybean and other linolenate oils for use in edible and industrial products.	Peoria, Ill.	Yes	11-B-1
N4 2-109	Evaluation of edible soybean oil products by chemical, physical, and organoleptic methods to provide basic information for increasing applicability in food uses.	Peoria, Ill.	Yes	11-B-3
N4 2-110(CA)	Preparation and evaluation of selected linseed oil derivatives for use in protective coatings to eliminate blistering, prevent yellowing, and improve tint retention.	Fargo, N. Dak.	Yes	12-B-3
N4 2-111	Exploration of new nitrogen- and sulfur-containing polymers derived from linseed oil to give products suitable for use in protective coatings.	Peoria, Ill.	Yes	12-B-2
N4 2-112(C)	Investigations on the mechanism of homogeneous catalysis by organometallic complexes for the selective hydrogenation and isomerization of soybean oil.	Urbana, Ill.	Yes	11-B-1
N4 2-113	Investigations on a pilot-plant scale of the selective hydrogenation of soybean oil with heterogeneous catalysts to develop large-scale methods for producing flavor-stable, liquid edible oil.	Peoria, Ill.	Yes	11-B-2
N4 2-114(Gr)	Photochemistry of linseed oil polymers on metal oxide substrates.**	Chicago, Ill.	No	
N4 2-115(Gr)	Investigations on flatus and gastrointestinal problems caused by ingestion of soybean protein foods, as related to processing.**	Urbana, Ill.	No	
N4 2-116(Gr)	A study of antinutritional factors in varieties of soybeans as related to processing for producing improved protein foods.**	Minneapolis, Minn.	No	
N4 2-117	Investigationson bifunctional compounds synthesized from soybean oil and on the polymers and other products derived therefrom.**	Peoria, Ill.	Yes	10-B-1

*Discontinued during reporting year.
**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
NU-O-O-1 (AID)	Engineering development of processes for converting soybeans to high-quality full-fat soybean flours for use in various developing countries of the world.	Peoria, Ill.	Yes	11-E-1
N5 5	New and replacement crops utilization investigations.			
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants, as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.*	Peoria, Ill.	Yes	13-A-4
N5 5-32 (Rev.)	Chemical survey of seed lipids from uncultivated domestic and foreign plants to discover sources containing economic amounts of industrially valuable constituents.	Peoria, Ill.	Yes	13-A-1
N5 5-33 (Rev.)	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	13-A-2
N5 5-44 (Rev.)	Chemical studies on nitrogenous components, other than enzymes, of crambe and other new oilseed meals as a basis for improved meal utilization for feed and industrial purposes.	Peoria, Ill.	Yes	16-A-2
N5 5-47	Engineering studies on a process for converting Crambe abyssinica seed and closely related new oilseeds into oil and detoxified meal for evaluating the utilization potential of these new oilseed crops.	Peoria, Ill.	Yes	16-B-1
N5 5-48(C)	Investigations on the preparation of omega-amino tridecanoic acid from crambe oil, the production of polyamides from it, and evaluation of the polymers for industrial uses.	Birmingham, Ala.	Yes	15-B-1
N5 5-49	Investigation of the enzyme systems, bitter principles, pigments and other selected constituents of defatted crambe and closely related seed meals considered pertinent to seed processing and meal utilization as feed.	Peoria, Ill.	Yes	16-A-1
N5 5-50	Investigations on the chemical modification of crambe oil, and of acids readily derived from it to prepare derivatives or chemical intermediates having properties desirable for industrial use.	Peoria, Ill.	Yes	15-A-1
N5 5-53	Evaluation, through compositional investigations and pulping studies, of the utility of various handling techniques for kenaf in the preparation of chemical pulps for use in production of printing, writing, tissue, and other fine papers.**	Peoria, Ill.	Yes	13-B-1
N5 5-54	Development of specific and convenient analytical methods for individual rotenoids, and study of the preparation and composition of rotenone-containing extracts from Tephrosia vogelii, to improve the prospects for commercialization of extracts from this species.**	Peoria, Ill.	Yes	13-A-3
NU P-1	Pioneering Laboratory for Microbiology Chemistry.	Peoria, Ill.	Yes	1-C-7

*Discontinued during reporting year.
**Initiated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
(10)	Cereal and forage crops.			
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find microorganisms suitable for fermentative processes of importance in cereal grain utilization.*	Allahabad, India	No	
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.	Allahabad, India	Yes	1-C-1
UR-A7-(10)-20	Investigations on the preparation and characterization of new copolymers of cereal starch with other polysaccharides by heating mixtures in the dry state, to provide basic information for the development of new starch products suited for industrial applications.	Ahmedabad, India	Yes	1-B-5
UR-A7-(10)-25	Investigations on the separation of grain sorghum proteins into homogeneous protein components, to provide basic information for further characterization and application studies.	Bangalore, India	Yes	9-A-2
UR-A7-(10)-75 (Rev.)	Investigation of the distribution of aerobic actinomycetes in India, with particular emphasis on their isolation, characterization, antibiotic production, and preservation, for placement in the Culture Collection of the Agricultural Research Service as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Lucknow, India	Yes	1-C-1
UR-A7-(10)-98	Investigations of methods for the chemical preparation and characterization of hydroxyethyl ethers of cereal starches prepared by partial replacement of specific hydroxyl groups to obtain new starch products with improved properties.	Ahmedabad, India	Yes	1-B-5
UR-A7-(10)-111	Studies on the isolation from natural plant gums of aldobi- and aldotriuronic acids to provide reference compounds for structural investigations on microbial polysaccharides of potential industrial significance that are produced from cereal grains.	Kanpur, India	Yes	1-C-8
UR-A10-(10)-27	Studies on the preparation and properties of graft copolymers of starch and dextrin obtained by reaction with vinyl monomers and epoxides, to provide a basis for increased industrial utilization of cereal grains.	Jerusalem, Israel	Yes	1-B-3
UR-A10-(10)-51	Fundamental studies on the mild oxidation of cereal grain starches by selected oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of modified starches of importance to their production and industrial use	Jerusalem, Israel	No	

*Completed project.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
UR-A11-(10)-17	Investigations to discover microorganisms that produce useful quantities of D-tartaric acid, and to explore laboratory processes for its production by fermentation of cereal grain products, as a basis for increasing the utilization of such products.	Tokyo, Japan	Yes	1-C-8
UR-A11-(10)-19	Investigations on the development of a polarographic method for determining aldehyde and ketone carbonyl groups in products derived from cereal grain starches to facilitate the characterization of such polysaccharides as a basis for increasing their utilization.	Sakai, Japan	Yes	1-A-3
UR-A11-(10)-22	Investigations to discover microorganisms that produce useful quantities of mevalonic acid, and to develop an efficient process for the economic production of mevalonic acid by fermentation of cereal grain products, as a basis for increasing the utilization of such products.	Tokyo, Japan	Yes	1-C-8
UR-E3-(10)-6	Rheological studies on aqueous dispersions of modified cereal starches and paper coating formulations containing starch-based adhesives, to provide fundamental information needed for the continued and increased utilization of starch in paper coating applications.**	Graz, Austria	Yes	1-B-7
UR-E4-(10)-2	Search for lytic enzymes of microbial origin with activity on cell walls of bacteria, actinomycetes, molds, and yeasts to provide a basis for the development of new fermentation processes for the increased utilization of cereal grains.	Liege, Belgium	Yes	1-C-3
UR-E15-(10)-32	Investigations on the conformation of glucopyranose rings in amylose corn starches and in linear and cyclic dextrins prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.*	Milan, Italy	Yes	1-A-3
UR-E19-(10)-18	Studies on the preparation of metal alkoxides of starch for use as intermediates in the synthesis of starch derivatives, to provide a basis for increasing the industrial utilization of cereal starches.	Delft, The Netherlands	Yes	1-B-2
UR-E21-(10)-11	Investigations on the fermentative production of itatartaric acid from glucose, sucrose, or molasses to provide new industrial outlets for these agricultural materials.	Lodz, Poland	Yes	1-C-8
UR-E21-(10)-34	Studies on carotene biosynthesis by the mold Blakeslea trispora, with emphasis on factors in spent mycelia of the mold that stimulate carotene production, to increase the yield of β-carotene obtained by fermentation of cereal grains.	Poznan, Poland	Yes	3-B-2

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
UR-E25-(10)-45	Investigation of the distribution of aerobic actinomycetes in Spain, with particular emphasis on their isolation, characterization, antibiotic production and preservation, for placement in the Culture Collection of the Agricultural Research Service as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Madrid, Spain	Yes	1-C-1
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.*	Newcastle upon Tyne, England	Yes	1-C-7
UR-E29-(10)-69	Fundamental studies on the nature and specificity of starch- and glycogen-debranching enzymes and the application of these enzymes to a study of the fine structures of amylopectins, amyloses, and glycogens of cereal grains, to provide a basis for increased utilization of cereal grains.	London, England	Yes	1-A-2
UR-E29-(10)-71	Investigations on the mechanism and structural changes involved in thermal, acid, or alkali degradation of cereal starches, to provide basic information for the development of new starch products suited for industrial applications.	Edinburgh, Scotland	Yes	1-B-4
UR-E30-(10)-1	Studies on the modification of cereal grain starches by physical treatment of granular starch under different conditions of moisture, temperature, and pressure in order to impart new paste properties as a basis for increased utilization of cereal grains.	Ljubljana, Yugoslavia	Yes	1-B-7
UR-S3-(10)-11	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncrosslinked starches, to create new markets and expand old markets for starch from cereal grains.*	Rio de Janeiro, Brazil	Yes	1-B-5
(10,40)	Cereal, forage crops, and oilseeds.			
UR-A11-(10,40)-10	Investigation of crosses of *Saccharomyces rouxii* isolated from the soybean fermentations, shoyu and miso, and an evaluation of their fermentative abilities in the above fermentation processes, as a basis for increasing the use of soybeans in fermented foods.	Noda-shi, Chiba-ken, Japan	Yes	11-D-3
(40)	Oilseeds			
UR-A6-(40)-1	Investigation of the various processes used in preparing Chinese cheese by the fermentation of soybean curd with *Mucor* and other fungi as a basis for increasing the foreign utilization of soybeans.	Taipei, Taiwan	Yes	11-D-4

*Completed project.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
UR-A7-(40)-21	Exploratory investigations of selected hydroxylated derivatives of linseed and safflower oils, to determine the feasibility of producing new industrial products from these oils.	Hyderabad, India	Yes	12-B-5
UR-A7-(40)-95	Laboratory studies on the fermentative production of microbial lipases that are useful in converting vegetable oils to products of higher value as a basis for increasing the utilization of soybean and linseed oils.	Baroda, India	Yes	10-C-1
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.*	Rehovot, Israel	Yes	11-A-3
UR-A10-(40)-20	Laboratory investigations on miso-type food products by fermentation of soybean meal products and cereal grains for use in Israeli foods.*	Ramat Gan, Israel	Yes	11-D-3
UR-A10-(40)-30	Investigations of the effect of processing conditions on the yield and quality of isolated soybean protein for use in Israeli-type foods, as a contribution to expanded utilization of soybeans.*	Haifa, Israel	Yes	11-E-2
UR-A10-(40)-92	Fundamental investigations on glycoproteins of soybean meal to provide information basic to increasing the utilization of soybean food and feed products.**	Rehovot, Israel	No	
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.*	Tokyo, Japan	Yes	11-D-3
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.*	Kawagoe, Saitama-ken, Japan	Yes	11-B-2
UR-A11-(40)-8	Isolation and determination of the flavor components of enzymatically or chemically modified soybean meal and proteins, and elucidation of their chemical and physical properties, to provide information basic to improving the flavor and thus increasing the utilization of soybeans.	Tokyo, Japan	Yes	11-B-4
UR-A11-(40)-11	Evaluation of United States soybean varieties as a material for producing fresh tofu to increase utilization in Japan.	Tokyo, Japan	Yes	11-D-2
UR-A11-(40)-13	Isolation, characterization, and quantitative determination of the sterols in soybeans to provide basic information for the evaluation and improvement of soybean meal and soybean products as foods and feeds.	Tokyo, Japan	Yes	11-A-3
UR-A11-(40)-14	Fundamental studies on color reversion of edible soybean oil to obtain information on its cause and prevention, as a means of increasing the utilization of soybean oil for food purposes in Japan.	Tokyo, Japan	Yes	11-C-1

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
UR-A11-(40)-21	Investigations on comparative production of shoyu (soy sauce) from defatted soybean meals obtained from United States and Japanese soybeans and processed by United States and Japanese methods, to provide data for the increased use of United States beans.	Tokyo, Japan	Yes	11-D-3
UR-A11-(40)-26	Basic investigations on the development of foods from enzymatically treated soybean protein concentrates, to increase the use of United States soybeans in Japan.	Tokyo, Japan	Yes	11-D-2
UR-A11-(40)-31	Studies on enzymatic hydrolysis of soybean oligosaccharides to provide information basic to increasing the utilization of soybean food and feed products.	Takamatsu, Japan	Yes	11-A-3
UR-A11-(40)-32	Evaluation of United States soybeans and processing conditions for manufacture of dried tofu.**	Tokyo, Japan	Yes	11-D-2
UR-E15-(40)-46	Studies on the effect of stereospecific polymerization catalysts on fatty esters from soybean and linseed oils, to provide a basis for increasing the industrial utilization of these vegetable oils.*	Milan, Italy	Yes	12-B-4
UR-E15-(40)-48	Synthesis and use of lipid-soluble metal chelates of Schiff bases as catalysts for the selective hydrogenation of soybean oil, to provide a basis for improving its flavor stability for edible use.*	Milan, Italy	Yes	11-B-1
UR-E21-(40)-6	Chromatographic determination of the glyceride composition of selected erucic-acid containing oils, to provide basic information important to their utilization.*	Warsaw, Poland	Yes	13-A-2
UR-E21-(40)-36	Synthesis of pure triglycerides of known structure, in which at least one acyl grouping is derived from a 22-carbon or 20-carbon monoenoic acid, as model compounds for research on seed oils from crambe and related cruciferous plants, to provide a basis for their industrial utilization.**	Warsaw, Poland	No	
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, interfacial tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.*	Granada, Spain	Yes	11-B-3
UR-E26-(40)-3	Compositional investigations of Swedish Cruciferae (mustard family) seeds to find strains with maximum erucic acid content in their oils and minimum content of glucosidic precursors of isothiocyanates and thiooxazolidones in their meals, to provide a basis for their utilization as industrial oilseeds in the United States.	Svalof, Sweden	Yes	13-A-1

*Completed project.
**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1966 to June 30, 1967 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
(50)	Sugar and miscellaneous crops.			
UR-E15-(50)-29 (Rev.)	Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.*	Rome, Italy	No	

*Completed project.

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